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— SECTION C —

BOTANICAL SCIENCES

Contents

Page

Physiologic Specialization of Oat Stem Rust in Canada—*M. Newton and T. Johnson* - - - - - - - - - - 201

Notes on Seed-Borne Fungi. II. *Alternaria*—*J. W. Groves and A. J. Skolko* - - - - - - - - - - - - - - - 217

Studies with *Bacillus polymyxa*. I. Some Factors Affecting the Fermentation of Wheat by *Bacillus polymyxa*—*H. Katznelson* - - - - - - - - - - - - - - - - 235

Studies with *Bacillus polymyxa*. II. Bacteriophage of *Bacillus polymyxa* in Relation to the 2, 3-Butanediol Fermentation —*H. Katznelson* - - - - - - - - - - - - - - 241

A Cultural and Cytological Investigation of a Two-Spored Basidiomycete, *Aleurodiscus canadensis* n. sp.—*A. J. Skolko* 251

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PHYSIOLOGIC SPECIALIZATION OF OAT STEM RUST IN CANADA[1]

BY MARGARET NEWTON[2] AND T. JOHNSON[3]

Abstract

An account is given of the results of studies on the physiologic specialization of *Puccinia graminis Avenae* Erikss. and Henn. in Canada since the inception of the work. Twelve physiologic races were identified from 2586 isolates studied during the period 1921 to 1943. The annual surveys of the prevalence of physiologic races during this period show that each year races 1, 2, and 5 have comprised the bulk of the oat stem rust in all parts of Canada. The predominance of these races has been greatest in the three Prairie Provinces, where barberry is virtually non-existent. In regions where barberry is present, other races of greater range of pathogenicity have been found more frequently than in these three provinces. The occurrence of such races, however, was sporadic until 1943, in which year races 8, 10, and 11 attained a wide distribution, apparently traceable to windborne urediospores from the south. There is evidence that the strains of these races present in 1943 remain in the uredial stage for much longer periods than do strains of the same races collected in previous years—a fact that may have favoured their spread in this year.

The role of barberry in the origination of generally virulent physiologic races is discussed. The possibility that such races may also originate by mutation is suggested by spontaneous pathogenic changes that occurred, in the greenhouse, in a culture of race 3 that gave rise to several cultures of race 7.

The effect of environment on the identification of physiologic races is demonstrated by the seasonal influences on the reaction of the variety Sevnothree to races 1 and 11. On this variety the characteristic type 1 is frequently replaced, in summer, by type x.

Experiments on the influence of temperature on the reaction of adult oat plants to stem rust showed that a constant temperature of about 80° F. does not appreciably affect the reaction of White Tartar and Richland, or of other varieties with the same type of resistance, but is capable of breaking down the resistance of certain varieties derived from the crosses Hajira × Joanette, Hajira × Banner, and Victoria × (Hajira × Banner).

Introduction

Although oat stem rust (*Puccinia graminis Avenae* Erikss. and Henn.) has been present in Canada annually for many years, its destructiveness to the oat crop is not easy to ascertain. Greaney (10) estimated that during the period 1929–1934 the average annual loss in Manitoba and Saskatchewan from oat stem rust amounted to 8,344,000 bushels. No data are known from

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which reliable estimates could be made of losses caused elsewhere in Canada, but from what is known concerning the distribution of oat stem rust in other parts of the country, it seems probable that very considerable losses have occurred in certain years. The annual surveys of the prevalence of plant disease in Canada, and other sources of information, show that during the 24-year period 1920–1943, oat stem rust has been present every year in all of the nine provinces of Canada, with the possible exception of British Columbia where it may have been absent in certain years. In Ontario, Quebec, and the Maritime Provinces, there is evidence that the outbreaks of oat stem rust are frequently associated with the presence of the common barberry (*Berberis vulgaris* L.). Such local outbreaks often cause considerable damage. In the Prairie Provinces, where barberry is practically absent, stem rust of oats is annually initiated by wind-borne spores carried by southerly winds, and the distribution of the rust towards the end of the growing season appears to be more uniform than in other parts of the country. Epiphytotics usually reach their greatest intensity in the Red River Valley, and the amount of rust diminishes in a westerly and a northerly direction. Severe damage to oats rarely occurs in western Saskatchewan or in Alberta. The northernmost point at which oat stem rust has been found is Gillam, Man., on the Hudson's Bay Railway, in approximately latitude 56°. It has not been found in the Peace River Valley of Alberta or in the agricultural areas lying east of Prince Rupert, B.C.

The specialization of stem rust of oats into a number of physiologic races assumes a particular importance in view of the fact that, as far as is known, no commercially grown variety in America or elsewhere is resistant to all these races. Until a decade ago, practically all oat varieties grown in Canada were completely or moderately susceptible to all the physiologic races that were prevalent in the country. The distribution of Anthony, in 1933, and of Vanguard, in 1937, provided Canadian farmers with oat varieties highly resistant to physiologic races 1, 2, and 5. These three races have made up the bulk of the oat stem rust prevalent in this country since studies on the specialization of this rust were first undertaken 22 years ago. The annual occurrence of races to which these varieties are susceptible, even if these races have not hitherto been widely distributed, is a potential threat to the new varieties, as one or more of these races may at any future time assume greater prevalence than they have assumed in the past. To avoid this danger, the plant breeder must, therefore, take such races into consideration in his breeding program, and, if possible, develop varieties that are resistant to all races that occur in the country. Considerable progress towards this end has already been made. Varieties that, under ordinary growing conditions, possess resistance to all known races are in existence at the Dominion Rust Research Laboratory, although the ones thus far produced do not appear to have sufficiently satisfactory agronomic qualities to warrant their distribution.

Annual Oat Stem Rust Surveys

Annual surveys of the prevalence of physiologic races of oat stem rust have been conducted in Canada since 1921. No uniform method of collecting the rust has, however, been in practice for the whole country. In the Prairie Provinces, field surveys have been made annually by the staffs of the laboratories of plant pathology at Winnipeg, Man., Saskatoon, Sask., and Edmonton, Alta. In these surveys, attempts were made each year to obtain collections from points satisfactorily distributed throughout the areas in which oat stem rust occurred. In the other provinces, collections were obtained through the co-operation of various persons, particularly the members of the staffs of the experimental farms and stations, laboratories of plant pathology, and agricultural colleges. Systematic surveys were not made, except in the Maritime Provinces from 1936 to 1942, and in Ontario and Quebec in 1939. From 1926 to 1929, and from 1936 to the present time, collections of rust were obtained from rust nurseries maintained at one or more of the experimental farms or stations in each province.

Until 1928, the surveys were directed by Dr. D. L. Bailey and Dr. W. L. Gordon, the latter continuing them until 1932, after which they were taken over by the writers.

Specialization of Oat Stem Rust and Distribution of Physiologic Races in Canada

That oat stem rust is specialized into several physiologic races was demonstrated in 1923 by Stakman, Levine, and Bailey (22), who described four distinct races and inferred the existence of a fifth. Bailey (1) continued this work and, in 1924, described a fifth race. These five races were distinguished by the infection types that they produced on three differential oat varieties, White Tartar, Richland, and Joanette Strain (see Key and Table I). Only three of the races (races 1, 2, and 5), however, were obtained from rust samples collected in North America. Race 3 occurred in collections from Sweden and South Africa, and race 4 only in a collection from Sweden. Bailey, who studied collections of Canadian origin, found races 1, 2, and 5 widely distributed in the Prairie Provinces. These three races resemble one another in their inability to infect heavily the differential varieties White Tartar and Richland, whereas race 3 infects White Tartar heavily and Richland lightly, and race 4 infects both varieties heavily. Until 1924, therefore, no races had been found in North America that were highly pathogenic to White Tartar or Richland, and it appeared that the pathogenicity of oat stem rust in America was more limited than in Europe and in South Africa.

In 1925, work on the specialization of oat stem rust in Canada was undertaken at the Dominion Laboratory of Plant Pathology, Winnipeg. A survey covering the whole of Canada showed (8) that, in 1925, races 1, 2, 3, 4, and 5 were present, as well as a sixth race (race 6), which was capable of attacking all of the differential oat varieties. Although the presence in Canada of races 3, 4, and 6, was thus demonstrated, they comprised only a

ANALYTICAL KEY FOR THE IDENTIFICATION OF PHYSIOLOGIC RACES OF *P. graminis Avenae* ON THE BASIS OF THEIR PATHOGENICITY ON THREE SELECTED DIFFERENTIAL VARIETIES OF OATS

Reaction of differential hosts	*Physiologic race*
White Tartar resistant	
Richland resistant	
Sevnothree resistant	1
Sevnothree mesothetic	5
Sevnothree susceptible	2
Richland mesothetic	9
Richland susceptible	
Sevnothree resistant	11
Sevnothree mesothetic	10
Sevnothree susceptible	8
White Tartar susceptible	
Richland resistant	
Sevnothree resistant	3
Sevnothree mesothetic	12
Sevnothree susceptible	7
Richland susceptible	
Sevnothree resistant	4
Sevnothree mesothetic	13
Sevnothree susceptible	6

TABLE I

INFECTION TYPES PRODUCED ON SEEDLING LEAVES OF THREE OAT VARIETIES BY 13 PHYSIOLOGIC RACES OF *Puccinia graminis Avenae*

Race	White Tartar			Richland			Sevnothree		
	Range			Range			Range		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
1	2−	3n+	2+	0;	1++	1	1−	2++	1±
2	2=	3n+	2++	1−	2+	1+	4=	4+	4
3	3	4+	4−	1	2++	1	0;	1	1−
4	4−	4++	4	4−	4++	4+	0;	1+	1
5	1++	3n+	2++	1−	2+	1++	1c	x++	x±
6	4+	4++	4+	4+	4++	4+	4+	4++	4+
7	3	4+	4	0;	2+	2=	3	4+	4−
8	0;	2+	2	3	4+	4	3	4	3++
9	2−	3n+	2±	x=	4n+	x+	3++	4++	4
10	1	2+	2	3	4++	4	x−	x++	x+
11	1	2+	2+	3	4++	4	1−	2++	1±
12	4	4+	4=	1	2+	1++	x−	x++	x+
13	3+	4+	4−	3+	4+	4−	x−	x+	x

EXPLANATION OF SYMBOLS:

0 — No uredia developed, but hypersensitive flecks usually present.
1 — Uredia minute, isolated, and surrounded by sharply defined necrotic areas.
2 — Uredia small, usually isolated, and surrounded by necrotic or sharply chlorotic areas.
3 — Uredia of medium size with a tendency to coalesce; true hypersensitiveness absent but a moderate degree of chlorosis present.
4 — Uredia large, numerous, and confluent, hypersensitiveness absent but chlorosis often present.
x — Uredia very variable; all the infection types—necrotic flecks, and uredia of types 1, 2, 3, and 4—may occur together on the same leaf.
(=), (−), (±), and (+) indicate quantitative variations in the types of uredial infection. A semicolon (;) indicates the presence of necrotic flecks. The letters (c) and (n) denote, respectively, chlorosis and necrosis surrounding the uredia.

small proportion of the isolates studied—about 2%. Gordon and Welsh (9), in 1932, reported the occurrence in Canada of three additional physiologic races, namely, races 7, 8, and 9. Race 7 was first found in 1928; race 8 in 1929*; and race 9 in 1930. All three of these races, as well as races 4 and 6, possess a wider pathogenic range, owing to their ability to attack either White Tartar or Richland, than do the commonly occurring races 1, 2, and 5. Despite their narrower range of pathogenicity, however, races 1, 2, and 5 remained the predominant races of oat stem rust to such an extent that, during the period 1925–1930, they made up 97.3% of all isolates. Since 1930, three additional races have been found by the writers to be present in Canada. Race 10, which had previously been discovered in the United States (3) under circumstances suggestive of origin from barberry, was first isolated in 1935; race 11, first recorded in Germany (12), was found in 1938; and race 12, not previously found elsewhere, was discovered in the latter year. Pathogenically, these three races are not strikingly different from some of those found earlier. Races 10 and 11 resemble race 8 in producing a light infection of White Tartar and a heavy infection of Richland; and race 12 resembles races 3 and 7 in its pathogenic behaviour on the same hosts (Table I). Race 13 which is included in Table I, has not yet been found in field collections, and has been obtained only from aecia on barberry inoculated artificially with race 3. The distribution of physiologic races during the period 1921–1943, according to geographical areas, is given in Table II.

The increase in the number of physiologic races collected in the field has, however, scarcely affected the predominance of races 1, 2, and 5 (Fig. 1). During the period 1931–1942, these comprised 95.6% of all isolates, the maximum figure being 100% in 1933 and 1934 and the minimum 90.2%, in 1942. The reason for the predominance of these three races is not known with certainty, though possibly, as suggested by Gordon (7), it is related to the fact that they are slower than any of the other races of oat stem rust in initiating the formation of teliospores and, therefore, produce urediospores over a longer period.

The other physiologic races continued to be of no particular consequence until 1943, when races 8 and 10, and to a certain extent, race 11, became commonly distributed in Manitoba and Saskatchewan, and apparently also in Eastern Canada. In that year, these races comprised 25.2% of all isolates. This figure should not be taken to indicate that these races made up 25.2% of all the oat stem rust present in Canada in 1943, because many of the rust collections were taken from Vanguard and other varieties that are resistant to races 1, 2, 3, 5, 7, and 12, and, consequently, there was little chance that these races would be present in collections from such varieties. When the computation is based on rust collections from varieties susceptible to all

** Race 8 was isolated twice in 1929—once from uredia collected at Nappan, N.S. and once from aecia on a barberry artificially inoculated at Winnipeg with overwintered telia collected on oats. It was collected also in 1932 and in 1934 on the plots of the Dominion Rust Research Laboratory at Winnipeg, but these records have not been accepted as authentic owing to the possibility that the race may have spread to the plots from the greenhouse where it was maintained in culture.*

TABLE II

DISTRIBUTION BY GEOGRAPHICAL AREAS OF PHYSIOLOGIC RACES OF *Puccinia graminis Avenae* COLLECTED ON CEREALS AND GRASSES FROM 1921 TO 1943. (FIGURES REPRESENT THE NUMBER OF TIMES EACH RACE WAS COLLECTED IN EACH GEOGRAPHICAL AREA)

Race	1921 – 23	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	Total
Maritime Provinces																						
1	–	–	–	–	–	5	–	–	1	–	–	–	–	–	–	8	6	1	–	2	1	24
2	–	–	2	5	5	4	4	2	1	–	3	–	1	5	13	9	3	5	4	3	5	74
3	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	2
4	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
5	–	1	2	19	8	8	4	9	10	–	2	1	6	9	13	23	11	13	25	6	3	173
7	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
8	–	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	2
9	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
10	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	1	1	2	–	6
11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
Ontario and Quebec																						
1	–	5	–	–	1	4	1	–	–	–	–	–	–	–	–	13	7	1	3	2	1	38
2	–	5	5	9	14	10	7	5	8	7	3	–	3	14	5	24	15	16	18	6	8	182
3	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	1	–	3
4	–	–	–	–	–	–	1	–	–	–	–	–	–	1	–	–	–	–	–	–	–	2
5	–	5	1	14	14	8	3	2	6	5	3	–	8	11	7	31	36	8	24	17	9	212
6	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
7	–	–	–	–	–	3	3	1	–	–	–	–	1	–	–	–	2	–	–	1	–	11
8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	1	3
10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	3	5
11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	2	–	–	4
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	2	2	4	3	12
Prairie Provinces																						
1	5	4	2	2	9	30	9	7	3	2	–	–	–	–	–	6	1	2	2	–	1	85
2	22	9	32	67	131	97	55	73	39	40	17	26	9	22	17	22	24	23	25	26	27	803
3	–	–	1	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
4	–	–	2	–	4	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
5	15	14	29	111	198	83	41	36	28	46	18	44	10	4	10	15	18	12	37	44	18	831
6	–	–	1	3	1	2	–	1	–	–	–	–	1	–	1	–	–	–	–	–	–	10
7	–	–	–	–	–	1	1	–	1	–	–	–	–	–	–	–	2	–	–	–	–	5
8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	2	12	16
10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	8	9
11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	2
British Columbia																						
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
2	–	–	–	–	1	2	–	1	3	–	–	–	–	–	2	–	–	2	3	4	1	19
3	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1
5	–	–	–	–	1	1	–	–	5	1	–	–	1	1	4	1	3	2	6	1	1	28
10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	1	3
Total	42	43	77	231	388	259	132	139	109	102	46	71	42	67	73	156	130	94	155	123	107	2586

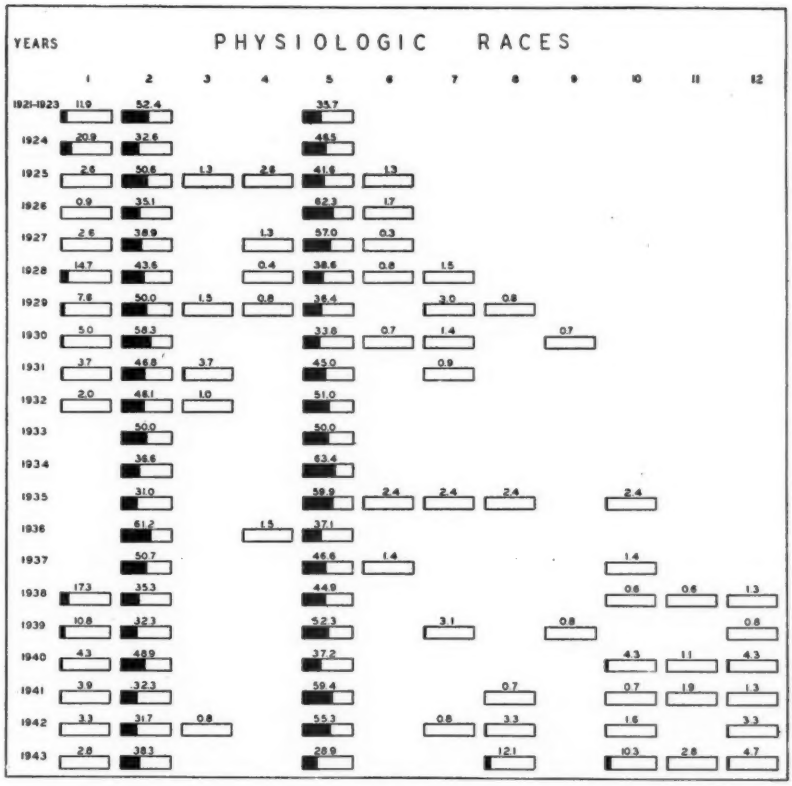

FIG. 1. *Annual frequency of occurrence of physiologic races of oat stem rust isolated in Canada during the period 1921–1943 expressed in per cent of the total isolates for each year.*

physiologic races, it is found that races 8, 10, and 11 make up only 13.2% of all isolates—a figure that gives a much nearer approximation of their relative prevalence, and agrees rather closely with the statement of Stakman *et al.* (25) that race 8 made up 12% of the racial isolates for the United States in the same year. As these three races attack Richland and all other oat varieties having the same stem rust reaction—such as the recently distributed varieties Vanguard and Ajax—any permanent increase in their prevalence would be a matter of grave concern.

No satisfactory reason can, at present, be given for the sudden increase in the prevalence of these races in 1943. Race 8 is known to have been present in Canada sporadically since 1929, without ever before having assumed any practical importance. Race 10 has also been present occasionally since 1935. Whatever part barberry may have played in the origin of these races, their distribution in Western Canada in 1943 was clearly attributable to wind-borne spores that presumably drifted in from the south.

The isolates of races 8, 10, and 11 that were collected in 1943 differ from those collected in earlier years in respect to the pronounced delay in teliospore formation. Gordon (7) showed that cultures of races 3, 4, 6, 7, 8, and 9 produced teliospores earlier than did cultures of races 1, 2, and 5. Similarly, it has been noted by the writers that cultures of races 8, 10, and 11, collected in previous years, remained in the uredial stage for only a short time before telia developed. When, however, cultures of these races collected in 1943 were studied, it was observed that telia were rarely produced on rusted seedling leaves of oats, as the leaves usually died before telia developed. A comparison was made of the time of telial formation in a culture of race 8 collected in 1943 and in a culture of the same race collected several years previously. Observations on infected seedling leaves of 10 oat varieties showed that, 30 days after inoculation, approximately 75% of the pustules of the earlier-collected race 8 had produced teliospores, whereas only the merest trace of teliospores could be found in the 1943 collection. If, as has been suggested (7, 18), a tendency to remain for long periods in the uredial stage without the production of telia is conducive to the spread and survival of a physiologic race, it may well be that, in this respect, the present strains of races 8, 10, and 11 have an advantage over those collected in previous years.

Nothing definite is known as to why teliospores developed so much later in the 1943 collections than in collections of the same races made in earlier years. There seems, however, to be no reason why races that develop telia early should not, by infection of barberry in nature, give rise to strains of the same races that develop telia later, that is to say, after a prolonged uredial period—a phenomenon that has actually been observed in studies on the artificial selfing of some races of oat stem rust*.

Seasonal Fluctuation in the Reaction of Oat Varieties to Stem Rust

It is now generally recognized by students of the rust fungi that factors in the environment, particularly temperature and light, exert a strong influence on the pathogenic characteristics of the rusts. With any given rust, the extent of this influence varies according to the host variety on which the rust is growing. Thus, in oat stem rust, the environment has a less pronounced influence on the infection types of physiologic races on the differential varieties White Tartar and Richland than on Joanette and Sevnothree. Waterhouse (26) showed that race 1 produced on Joanette a type 1 infection in winter, a type x in spring and fall, and a type 3+ in summer. Gordon (6) showed that certain physiologic races have a tendency to produce a type 1 infection or even necrotic flecks on seedling leaves of Joanette at a temperature of 57° to 60° F., and a type x, or even a type 3 or 4 at a temperature of 75° to 77° F. He concluded that variations in temperature that occur under ordinary greenhouse conditions were capable of affecting the reaction of this host. This conclusion is confirmed by observations that have been made by the

* *Unpublished data of the authors.*

writers over a period of more than two years on the infection types produced by certain physiologic races of oat stem rust on the differential hosts Victory, White Tartar, Richland, and Sevnothree.

From July, 1940, until February, 1943, races 1, 3, and 11 were kept in continuous culture in a greenhouse without thermostatic control of temperature but furnished, in summer, with slat blinds to reduce somewhat the excessively high temperatures caused by direct sunlight. The fluctuations in the infection types produced by these races on the varieties Victory, White Tartar, and Richland during this period were almost negligible, whereas the fluctuations in the infection types on Sevnothree were quite marked and followed, for all three races, a consistent course, which is shown diagrammatically for two of these races in Fig. 2. The normal infection produced by these races on Sevnothree is type 1. During fall, winter, spring, and early summer, this was the type almost invariably encountered. At midsummer, when the greenhouse temperature rose to its highest point for the year, the type of infection was usually either 2 or x. At this time, races that normally produce an x infection have a tendency to produce a 3 or 4 type of infection. Races that normally produce a 3 or 4 type of infection on Sevnothree appear to be little influenced by seasonal changes in the environment.

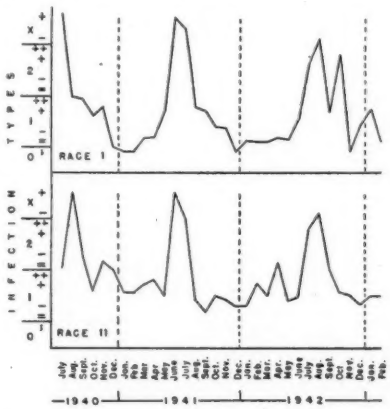

FIG. 2. *Seasonal effect over a period of 32 months on the infection type produced by physiologic races 1 and 11 of oat stem rust on the variety Sevnothree. For description of infection types and their quantitative variations see explanation of symbols, Table I.*

The range of seasonal fluctuation in the infection type produced by race 1 on Sevnothree, in this series of observations, was not quite so great as that recorded for the same race by Waterhouse. Nevertheless, it was great enough to make it difficult in the warm weather of summer, when the mean daily temperature of the greenhouse rose above 70° F., to distinguish race 1 from race 5; or, in fact, to distinguish any race that should produce on Sevnothree a type 1 infection from a race that should produce a type x. For accurate determination of such races, it is essential either to prevent an

excessive rise in greenhouse temperature or to avoid the indentification of races during the hottest part of the summer.

The Influence of Temperature on the Rust Reaction of Adult Plants

It has been shown (15) in tests conducted in the greenhouse, that a constant temperature of about 80° F. will induce susceptibility to wheat stem rust (*Puccinia graminis Tritici* Erikss. and Henn.) in the adult plants of some wheat varieties that at lower temperatures are highly resistant or immune to this rust. That a high greenhouse temperature may exercise a similar influence on the reaction to oat stem rust of certain oat varieties is evident from tests that have been carried out on adult oat plants. In December, 1941, and again in March, 1942, several oat varieties were inoculated, at the time of heading, with urediospores of physiologic races 5, 6, 8, and 12. After 24 hr. in a moist chamber, they were placed in greenhouses kept at three different temperatures: a constant low temperature of just under 60° F.; a constant high temperature of about 80° F.; and a moderately low temperature of slightly under 65° F.

The varieties tested included White Tartar and Anthony, which, in the seedling stage, are moderately resistant to races 5 and 8 but susceptible to races 6 and 12; Richland, Vanguard, Exeter, Ajax, and Vicland, which are resistant to races 5 and 12 but susceptible to races 6 and 8; and three varieties recently developed at the Dominion Rust Research Laboratory, R.L. 811 (Hajira × Joanette), R.L. 524 (Hajira × Banner), and R.L. 1276 (Victoria × R.L. 524). Of the three last-mentioned varieties, R.L. 811 is, in the seedling stage, highly resistant to all physiologic races of oat stem rust; and R.L. 524 and R.L. 1276 are resistant to all races except race 6 to which their reaction varies from moderate resistance to moderate susceptibility.

The data recorded in Table III show that whatever resistance is possessed by the varieties White Tartar, Anthony, Richland, Vanguard, Exeter, Ajax, and Vicland is relatively stable at the temperatures investigated. The resistance of the three new varieties—R.L. 811, R.L. 524, and R.L. 1276—showed much less stability. Indeed, in the test conducted in December, the high resistance shown by R.L. 811 to races 5, 6, 8, and 12 at low temperatures broke down completely at a constant temperature of from 76° to 79° F. In the second test, conducted in March, the breakdown at the high temperature was complete for race 6, but involved only a shift from immunity to resistance for the other three races. The other two varieties, R.L. 524 and R.L. 1276, included only in the second test, behaved in a somewhat similar manner. The reason for the greater degree of susceptibility in the December test is not known definitely, though, as poor conditions of light are known to promote increased susceptibility to stem rust of wheat (11, 14), it possibly was associated with the poorer conditions of light prevailing at that time of year.

TABLE III

MEAN REACTION OF LEAVES AND SHEATHS OF ADULT PLANTS OF 10 OAT VARIETIES TO FOUR PHYSIOLOGIC RACES OF OAT STEM RUST AT THREE TEMPERATURES IN THE GREENHOUSE

Variety	1st Test—December, 1941					2nd Test—March, 1942				
	Temperature, °F.	Race				Temperature, °F.	Race			
		5	6	8	12		5	6	8	12
White Tartar	58 – 59	MR	MS	MR	S	58 – 59	MR	S	MR	S
	62 – 63	MR	MS	MR	S	62 – 64	MR	S	MR	S
	76 – 79	MR	MR	MR	S	80 – 83	MR	S	MR	S
Anthony	58 – 59	MR	MS	MR	S	58 – 59	MR	S	MR	S
	62 – 63	MR	MS	MR	S	62 – 64	MR	S	MR	S
	76 – 79	MR	MR	MR	S	80 – 83	MR	S	MR	S
Richland	58 – 59	R	MS	S	R	58 – 59	R	S	S	R
	62 – 63	R	S	S	R	62 – 64	R	S	S	R
	76 – 79	R	S	S	R	80 – 83	MR	S	S	R
Vanguard	58 – 59	R	MS	S	R	58 – 59	R	S	S	R
	62 – 63	R	MS	S	R	62 – 64	R	S	S	R
	76 – 79	R	MS	S	R	80 – 83	R	S	S	R
Exeter	58 – 59	R	MS	S	R	58 – 59	R	S	S	R
	62 – 63	R	MS	S	R	62 – 64	R	S	S	R
	76 – 79	R	MS	S	R	80 – 83	R	S	S	R
Ajax	58 – 59	R	S	S	R	58 – 59	R	S	S	R
	62 – 63	R	S	S	R	62 – 64	R	S	S	R
	76 – 79	R	MS	S	R	80 – 83	R	S	S	R
Vicland	58 – 59	R	MR	S	R	58 – 59	R	S	S	R
	62 – 63	R	MR	S	R	62 – 64	R	S	S	R
	76 – 79	R	S	S	R	80 – 83	MR	S	S	R
R.L. 811 (Haj. × Joan.)	58 – 59	I	I	I	I	58 – 59	I	I	I	I
	62 – 63	I	I	I	I	62 – 64	I	I	I	I
	76 – 79	S	S	S	S	80 – 83	R	S	R	R
R.L. 524 (Haj. × Banner)						58 – 59	R	R	R	I
						62 – 64	R	R	R	R
						80 – 83	MR	S	MR	MR
R.L. 1276 (Victoria × R.L. 524)						58 – 59	R	R	I	I
						62 – 64	R	R	I	R
						80 – 83	R	S	R	R

NOTE: I = *Immune;* R = *resistant;* MR = *moderately resistant;* MS = *moderately susceptible;* S = *susceptible.*

As none of these varieties has shown any pronounced breakdown of resistance to stem rust under field conditions, it may be that no great significance should be attached to susceptibility in the greenhouse under conditions of constant high temperatures. Nevertheless, it is clear that the stability of resistance to oat stem rust varies greatly in different varieties, and, evidently also, according to the races attacking them.

Mutation

In giving consideration to the causes of variation in any organism, the possibility that mutation may play a part of some importance should not be overlooked. In the rusts, a number of apparently spontaneous changes regarded as mutations have been placed on record. These include changes in urediospore colour (17, 26), changes in pathogenicity (4, 19, 21, 23), and changes in both urediospore colour and pathogenicity (16, 20).

Probably the most striking and conclusive evidence for mutation affecting the pathogenicity of a rust is that given by Gassner and Straib (4), who described a series of recurrent mutations in *Puccinia glumarum* (Schmidt) Erikss. and Henn., resulting in identical mutants of greater infectivity than the original race from which the mutations arose.

Ordinarily, it is difficult to prove that any apparent change of pathogenicity in a rust is the result of mutation, unless the mutation produces a physiologic race previously unknown. If the suspected mutant is a race already known, and present in the locality concerned, it is usually thought safer to assume that an accidental admixture of the other race has taken place rather than that a mutation occurred. The case for mutation, however, is greatly strengthened if it is possible to demonstrate a recurrence over a period of time of the same pathogenic change, as Gassner and Straib were able to do.

Recently, a race of oat stem rust that had been kept in pure culture for many years has given rise to a series of pathogenic changes that have been interpreted as mutations. The culture in question was an isolate of race 3 that was kept in culture from 1933, with intervals of storage in a refrigerator, until July 1940, when it was placed permanently in the greenhouse. From that time onwards, the differential varieties were infected with it at intervals of about three weeks. Infection types were normal, with the exception of minor seasonal fluctuations, until early in December, 1941, when an x* type of infection was produced on the variety Sevnothree in place of the usual necrotic flecks or 1 type of pustule. Infection types were normal in the next uredial generation, which developed late in December. In January, 1942, the reappearance of an x type on Sevnothree led to a suspicion of an admixture of another race. Cultures were then established from three isolated uredia on the variety Victory with the object of purifying the race. Two of these produced a 4 type of infection on Sevnothree but were otherwise identical with race 3. These two cultures were therefore regarded as race 7 and were discarded. The third culture produced necrotic flecks on Sevnothree and was therefore thought to be a pure culture of race 3. The next inoculation with this culture, however, produced an x infection on Sevnothree and on Joanette, a variety formerly used in place of Sevnothree. In the meanwhile, the original culture kept on producing an x type of infection on both Sevnothree and Joanette. Attempts were then made to establish cultures from the 1 type pustules on Joanette and Sevnothree, on the supposition that

* *In the* x *type of infection, all the infection types—necrotic flecks, and pustules of types 1, 2, 3, and 4—may occur together on the same leaf.*

pustules of this type would give rise to cultures of race 3. On May 5, 1942, inoculations were made from eight uredia that conformed closely to the 1 type. Contrary to expectation, however, all of the eight cultures derived from them produced for the most part 3 and 4 types of infection on Sevnothree and are therefore to be regarded as cultures of race 7. One of these cultures, and also the original culture from which it was derived, have since been kept in continuous culture in the greenhouse without any observable change except the occasional production on Sevnothree of an x type of infection.

Here, the case for mutation rests on the failure to recover race 3 through isolates derived from segregated pustules that would be expected to yield that race—a process that ordinarily involves no great difficulty. It could not be simply a matter of an admixture of race 7, because that race does not produce 1 type infections on Sevnothree. Neither does it seem explainable on the basis of an admixture of race 12, which does, at times, produce 1 type pustules on Sevnothree. Inoculation from such pustules would presumably have given rise to cultures of race 12 rather than to cultures of race 7. It seems, therefore, justifiable to conclude that a process of recurrent mutation, commencing in December and continuing until next spring, led to the gradual displacement of race 3 by race 7, which differs from race 3 only in its greater pathogenicity on the variety Sevnothree.

Discussion

As the uredial stage of *Puccinia graminis Avenae* is believed not to overwinter in Canada, or at least to any appreciable extent, the annual recurrence of this rust seems dependent on wind-borne urediospores carried north by southerly winds in early summer, and such aeciospores as develop on barberry from infection arising from overwintered teliospores.

In the Prairie Provinces, barberry is virtually absent, and hence the early arriving inoculum is almost, if not entirely, composed of wind-borne spores that drift up from the Great Plains region farther south. As already pointed out, the distribution of oat stem rust in the Prairie Provinces is usually rather uniform but diminishes gradually in a westerly and a northerly direction.

In the settled regions of Ontario, Quebec, and the Maritime Provinces, there occur numerous locations of common barberry. Local outbreaks of stem rust are known to have spread from many of these. As oats is the predominant cereal crop in all these provinces, stem rust of oats is unquestionably of greater economic importance than stem rust of wheat or of rye. Surveys that have been made, particularly by Mr. I. L. Conners and Mr. S. F. Clarkson, seem to indicate that oat stem rust, though frequently severe in certain localities, is less uniformly distributed than in the Prairie Provinces. It seems probable that wind-borne spores play a smaller part in the spread of oat stem rust in the former provinces than in the latter, and that barberry is responsible for many of the local outbreaks of rust, some of which have, in fact, been directly traced to it. The following quotation from a report by Conners (2), after a survey of the Maritime Provinces, in 1937, is indicative of the role of barberry in that region.

"Traces of stem rust (of oats) were found in 60, and readable percentages in 44 out of 233 fields examined in the Maritime Provinces. While traces of stem rust began to be fairly common after August 16, severe outbreaks of stem rust were found as early as August 4. Several of these were investigated and in every instance the outbreaks centred about plantings of barberry. Some of the plantings were single bushes, others were hedges. It was estimated that the average damage was 40% within half a mile of each planting and it was nearly 100% in the first 100 yards. The damage was negligible elsewhere."

Quite apart from its ability to produce local outbreaks of rust the barberry may play a role in the origination or spread of physiologic races capable of attacking oat varieties now regarded as resistant. So long as the varieties grown are not resistant to all physiologic races of the rust, there is always a chance that the races capable of attacking them may assume predominance; and, so long as barberry is present, there is a possibility of such races being produced.

Theoretically, there is no reason why races more virulent than the common races 1, 2, and 5, should not develop from infections produced on barberry by these races, since it has been shown (13) that, in heterozygous races, the ability to produce a heavy type of infection on some oat varieties is recessive to the ability to produce a weaker infection type. In the selfing of such a race or in the crossing of two such races, the recessive genes may come together to produce strains of rust with the heavy type of infection. Actually, a culture of race 1, which was produced synthetically by crossing race 11 with race 7, has been used to infect barberry with the result that the aeciospores gave rise to uredial cultures of races 1, 3, 4, and 11*. Race 1, which attacks both the varieties White Tartar and Richland weakly, produced, therefore, after selfing on barberry, race 3, which attacks White Tartar heavily, race 11, which attacks Richland heavily, and race 4, which attacks both varieties heavily. Similarly, it was shown (9, 13) that race 8, which attacks White Tartar weakly, may infect barberry and produce aeciospores that give rise to uredial cultures of race 6, which attacks White Tartar heavily.

There can, consequently, be no doubt that barberry can function in the production of races capable of attacking White Tartar and Richland heavily. As most of the recently developed oat varieties resemble one or the other of these two varieties in respect to their reactions to oat stem rust, it follows that barberry is a factor that cannot be neglected in any program of breeding new varieties.

It is not easy to estimate how important a part barberry is playing in the origin and dissemination of the more virulent races of oat stem rust. Only physiologic races 2 and 5 were isolated by Stakman *et al.* (24) from six aecial

* *Unpublished experimental results of the authors.*

collections studied during the period 1920–1932. Similarly, Gordon (unpublished information), in 1928, isolated only races 2 and 5 from five collections of aecia from barberries growing at Ottawa, Ont. He, however, obtained races 1 and 3 from aecia on barberry leaves collected in the following year at Guelph, Ont. and isolated races 2, 3, 7, and 8 from barberries inoculated artificially with telial material collected on oats at Winnipeg, Man. (5). In a study by the authors of collections of aecia from eight separate localities in Eastern Canada in 1943, only two yielded isolates of oat stem rust. One was identified as race 2, the other as race 8. A study of the physiologic races present in 32 collections of oat stem rust made from 1937 to 1942 near barberry bushes in Eastern Canada, resulted in the identification of 3 isolates of race 1, 11 of race 2, 21 of race 5, and 1 of race 10. It may here be recalled that race 10 was originally discovered in a barberry area (3). The occurrence of race 8 on two separate occasions in cultures derived from aecia as well as the isolation of race 10 from a collection made in the proximity of barberries suggests the importance of barberry in the initiation of these races.

The data on the physiologic races present in non-barberry and barberry areas of the country are also suggestive. The only region of Canada in which barberries may be said to be absent, or virtually absent, is the three Prairie Provinces. During the period 1921 to 1942, races other than 1, 2, and 5 have constituted 1.8% of all isolates from this region. For the regions where barberry is present the figures are: the Maritime Provinces, 5.1%; Ontario and Quebec, 7.5%; British Columbia, 8.0%. Occasionally there occur other evidences of the possible origin of new or unusual strains of this rust from barberry such as the isolation by Gordon (7) of strains of oat stem rust with orange uredia from a barberry inoculated with teliospores collected in the field and the collection in Prince Edward Island, in 1942, of a culture of race 2 with gray urediospores. As strains with gray uredia are rarely found except in rust derived from aeciospores, this strain is likely to have originated from barberry.

Granting the importance of barberry in the production of new strains of rust, it does not necessarily follow that the removal of all barberry would preclude pathogenic changes in the rust organism; for it has been demonstrated that pathogenic changes in the rusts may occur through mutation. No definite information is at present available on the reasons for the sudden increase that occurred in 1943 in the prevalence of races 8, 10, and 11, though the tendency in these races for the uredial stage to persist for longer periods than in the same races collected in earlier years, may help to explain their wider distribution in that year. The possibility of future changes in the pathogenicity of the rust necessitates an adequate annual survey of physiologic races and a continued vigilance on the part of plant breeders in the development of varieties resistant to the races present.

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NOTES ON SEED-BORNE FUNGI

II. *ALTERNARIA*[1]

By J. W. Groves[2] and A. J. Skolko[2]

Abstract

Seven species of *Alternaria*, of which six are definitely pathogenic, have been isolated from agricultural seeds. Cruciferous plants are attacked by three distinct species; *A. Brassicae* (Berk.) Sacc., *A. oleracea* Milbr., and *A. Raphani* n. sp. The latter has been isolated only from radish seeds. Two species occur on carrots, *A. Dauci* (Kühn) n. comb., and *A. radicina* Meier, Drechsler, and Eddy. A species close to *A. Dauci* has been isolated from flax and is described as *A. linicola* n. sp. The name *A. tenuis* auct. is used to include the generally saprophytic forms that have been isolated from seeds of a wide variety of plants. They exhibit many variations in cultural characters, spore markings, etc., but all have spores falling within the range 20–50 × 10–16 μ.

The genus *Alternaria*

The genus *Alternaria* is used here in the sense of Elliott (7), Bolle (3), Wiltshire (29), and others, to include the fungi with obclavate, coloured, muriform spores borne in chains. This is the traditional concept of the genus but Wiltshire (29) has pointed out that a strict interpretation of the International Rules of Nomenclature would require that *Alternaria* be dropped and that all of the species be transferred to *Macrosporium*. The chief distinction between *Alternaria* and *Macrosporium* has rested on whether or not the conidia are borne in chains. Elliott (7) pointed out that a more fundamental difference was to be found in the shape of the spores, since many species formed chains very rarely. He distinguished two main types of spores; those that are clavate, tapering to the distal end and becoming more or less drawn out into an attenuated beak, and those that are packet-shaped or sarcinaeform, more or less constricted at the median septum and having both ends rounded. He concluded that all species with spores of the first type would form chains under suitable conditions and should be referred to *Alternaria*. It appeared from the descriptions of the foundation species of *Macrosporium* that most of them came within this first group, and that none belonged to the second group. He was of the opinion therefore that species of the second type should probably go to *Stemphylium*. Wiltshire (29, 30) was in agreement with the distinction pointed out by Elliott and made a careful study of the four foundation species of *Macrosporium* that revealed that three of them were *Alternaria* species and the fourth was a *Clasterosporium*. He pointed out that the genus *Macrosporium* was established by Fries in 1833 in the Systema Mycologicum, whereas *Alternaria* was established by Nees in 1817 but was not recognized by Fries in the Systema and was not used again until taken up by Chevallier

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in 1836. Consequently, according to the International Rules, *Macrosporium* is the valid genus for the species with clavate spores borne in chains and *Alternaria* should disappear. He showed conclusively that the species with sarcinaeform spores not borne in chains should go to *Stemphylium*.

However Wiltshire has urged that to reverse the traditional concept of *Macrosporium* from having the spores borne singly to having them borne in chains would lead to great confusion, and that it would be preferable to conserve *Alternaria* Chevallier against *Macrosporium* Fries. With this proposal we are in complete agreement. *Alternaria* would then be retained in its traditional sense and *Macrosporium* would disappear, or as Elliott suggested, might be used for the fungi now placed in *Clasterosporium*.

Alternaria tenuis auct. sensu Wiltshire. Trans. Brit. Mycol. Soc. 18 : 157. 1933.

Wiltshire (29) has discussed the status of the name *A. tenuis*. From his paper it is clear that this is the name given by Nees to the species on which he based the genus *Alternaria*. In the Systema Mycologicum, Fries did not recognize the genus *Alternaria* and described Nees's fungus under the name *Torula alternata*. It would seem that Fries changed Nees's specific name for no apparent reason, nevertheless under the International Rules, *alternata* is the valid specific name for Nees's fungus.

In 1840 Corda illustrated a fungus that he identified as *A. tenuis* Nees. However Wiltshire has pointed out that there are certain discrepancies between Corda's and Nees's figures that indicate strongly that Corda did not have Nees's fungus. Nevertheless Corda's figure has become the basis of the modern conception of *A. tenuis*. This conception was defined by Mason (14) as comprising all forms "whose spores are obclavate, borne in long chains, and the majority of whose spores have 3 to 5 cross septa and, especially in culture, fall within the limits 20–50 × 10–14 μ".

We therefore have the situation that *A. tenuis* Nees, which is generally stated to be the type of the genus *Alternaria*, is today unknown, and while Nees's figure may be admitted as representing a fungus that we would place in *Alternaria*, it is doubtful if the species could be recognized with certainty. Furthermore if it could be recognized it would have to be designated by the specific name *alternata* and a new combination in *Alternaria* created for it.

The traditional concept of *Alternaria tenuis* is based on Corda's fungus but his name is invalid because it must be regarded as having been based on Nees's type and his own use of it, the result of a misdetermination.

Thus the fungus that commonly goes under the name of *A. tenuis* is actually without a valid name. There is no doubt that this fungus has been described many times under many different names and it will be very difficult to determine what its correct name should be. In the meantime Wiltshire has suggested that for convenience, fungi that fall within Mason's characterization, which is fundamentally based on Corda's conception, should be designated as *A. tenuis* auct.

Accepting this suggestion we find that *A. tenuis* is the fungus most commonly isolated from seeds. The cultures, both from the same and different hosts, vary considerably in appearance (Figs. 1, 2, 4) but the range of variation is much the same in all. Aerial mycelium is usually fairly abundant, varying from loose, open, and cottony to close, tufted, and woolly in appearance, whitish to grayish, greenish to olivaceous, or brownish to almost black. On malt agar the cultures are usually more greenish and on pea agar more grayish. The amount of sporulation is also very variable, either scant or copious. Sometimes the conidiophores are more or less aggregated into tufts, sometimes evenly distributed over the colony. The conidiophores are olive-brown, septate, simple or branched, very variable in length, 3–6 μ in diameter. The conidia (Figs. 12, 19) are light olive-brown to dark brown, smooth or warty, usually with three to five cross septa and with longitudinal septa in the second and third cells, obclavate with rather short beaks and borne in long chains, (16)–20–50–(70) × (7)–10–16–(20) μ. It should be noted that these spores are described as they occur in culture for in nature they appear to have proportionately longer beaks.

This species has been isolated from the following seeds: *Agropyron cristatum* J. Gaertn. (crested wheat grass), *Allium cepa* L. (onion), *A. porrum* L. (leek), *Apium graveolens* L. var. *dulce* DC. (celery), *Asparagus officinalis* L. var. *altilis* L. (asparagus), *Avena sativa* L. (oats), *Beta vulgaris* L. (beets, mangels), *B. vulgaris* L. var. *cicla* L. (Swiss chard), *Brassica napobrassica* Mill. (swedes), *B. napus* L. (rape), *B. oleracea* L. var. *botrytis* L. (broccoli, cauliflower), *B. oleracea* L. var. *capitata* L. (cabbage), *Brassica rapa* L. (turnip), *Bromus inermis* Leyss. (brome grass), *Capsicum annuum* L. (pepper), *Cucumis melo* L. (muskmelon), *C. sativa* L. (cucumber), *Cucurbita maxima* Duch. (squash), *C. Pepo* L. (pumpkin, vegetable marrow), *Dactylis glomerata* L. (orchard grass), *Daucus carota* L. var. *sativa* DC. (carrot), *Festuca elatior* L. (meadow fescue), *F. rubra* L. (red fescue), *Glycine max* Merr. (soybean), *Helianthus annuus* L. (sunflower), *Hordeum vulgare* L. (barley), *Lactuca sativa* L. (lettuce), *Linum usitatissimum* L. (flax), *Lycopersicon esculentum* Mill. (tomato), *Medicago sativa* L. (alfalfa), *Melilotus alba* Desr. (sweet clover), *Pastinaca sativa* L. (parsnip), *Petroselinum hortense* Hoffm. (parsley), *Phaseolus coccineus* L. (scarlet runner beans), *P. vulgaris* L. (beans), *Phleum pratense* L. (timothy), *Pisum sativum* L. (peas), *Raphanus sativus* L. (radish), *Secale cereale* L. (rye), *Setaria italica* (L.) Beauv. (millet), *Spinacia oleracea* L. (spinach), *Taraxacum kok-saghyz* Rod. (Russian dandelion), *Trifolium hybridum* L. (alsike), *T. pratense* L. (red clover), *Triticum aestivum* L. (wheat), *Vicia faba* L. (broad beans), *Zea mays* L. (corn).

The above list of plants comprises all whose seeds we have tested by the plating method. This would indicate that the list might be extended almost indefinitely by the examination of other wild and cultivated plants. Likewise the geographic range is very wide as we have isolated this species from seeds produced in Canada from British Columbia to Prince Edward Island, and from

imported samples from California, Minnesota, Michigan, New York, and Connecticut, and from England, The Netherlands, Denmark, and Russia. The locality in which the imported seed samples were grown is not known with certainty, the only indication being the address of the firm from which they were purchased. Thus the concept of *A. tenuis* is of a species of world wide distribution of which the diagnostic character is the spore, occurring on all sorts of plants, and possibly other organic materials.

It is apparent from the descriptions of *Alternaria* species recorded in the literature, and from the examination of exsiccati, that many fungi that would fall within this concept have been described as distinct species, often chiefly on the basis of their occurrence on different hosts. The use of the host plant for distinguishing species of fungi, while undoubtedly justified in some cases is of doubtful value in a fungus such as *A. tenuis*, which does not appear to be specialized in its host relationship and in which the range of variation observed occurs equally on many plants.

As noted above, the isolates referred to this species show great variability, and if extreme types were taken it might be considered that the differences were enough to warrant specific rank. When, however, many hundreds of isolates are encountered from the variety of seeds listed above, and they are found to exhibit all gradations of difference, it is impossible to draw any specific lines. All attempts to find morphological characters that seemed to be stable and diagnostic have been unsuccessful.

It is to be expected that physiological differences will be found in different isolates. These differences may be manifested as varying degrees of pathogenicity. Roberts (23) found an *Alternaria* of the *tenuis* type to be a facultative parasite on apple leaves, capable of enlarging spots initiated by other fungi. Dey (6) described a disease of flax in India caused by an *Alternaria* of the *tenuis* type. Warner (26) described a disease of tomato fruits of which the causal fungus was an *Alternaria* of the *tenuis* type. Carpenter (5) found an *Alternaria* of the *tenuis* type to be the predominating fungus in apples affected with mouldy core. McClellan (13) described a seedling blight of castor bean caused by an *Alternaria* of the *tenuis* type. Other examples could be cited. Pathogenic strains are more likely to attract attention but undoubtedly other sorts of physiological differences occur also. Ledingham and Adams (12) found that 33 of our isolates of *A. tenuis* varied widely in their ability to decompose sulphite liquor.

The taxonomic significance of these differences is still obscure. Young (31) designated 16 physiologic forms of *Alternaria tenuis* and stated that they were clearly distinct and were separated on the basis of origin, cultural characters, and pathogenicity, but the diagnostic characters of each form are not clear and it does not appear possible that they could be recognized again.

In *A. tenuis* we have not, as yet, any acceptable basis for the separation of physiologically different strains. In the parasitic species of fungi where a great deal of work has been done on the differentiation of strains by means of the host relationships and pathogenicity, the recognition of these physiologic

forms is on a sound and workable basis. In other groups such as the yeasts, much work has been done on the physiological differentiation of strains on the basis of their reactions to various sugars, etc. But there are other fungi of generally saprophytic habit, wide geographic distribution, and ubiquitous host range, in which intensive studies of the physiology of a large number of strains have not been made. Outstanding examples of such fungi besides *A. tenuis* are, *Pleospora herbarum* Rabh., *Botrytis cinerea* Pers., and *Cladosporium herbarum* Fr. In the present stage of our knowledge any attempt to classify strains of these fungi on physiological characters would appear to be premature.

The perfect stage of *A. tenuis* is probably a *Pleospora* species in which the ascospores have three to five cross septa, rather than seven cross septa as in *P. herbarum*. The evidence for this connection rests chiefly on the work of Brefeld (4) and Klebahn (10) both of whom obtained *Alternaria* species of the *tenuis* type in cultures originating from ascospores. As far as we can ascertain no one has succeeded in obtaining the *Pleospora* stage in cultures originating from conidia and we have seen no evidence of the development of a perfect stage in any of our cultures. According to Mason (14) this *Pleospora* species is usually considered to be *P. infectoria* Fckl.

Alternaria Brassicae (Berk.) Sacc. Syll. Fung. 4 : 546. 1886.
Macrosporium Brassicae Berk. In Smith's Engl. Fl. 5: 339. 1836.

This name is used for the fungus causing the gray leaf spot of crucifers. It has long been known that, on crucifers, there were at least two parasitic species of *Alternaria* that could be distinguished by their spore size as well as by the symptoms produced on the host plant. However there has been a great deal of confusion in the literature with respect to the nomenclature of these fungi, probably originating with a misdetermination by Saccardo (25).

Macrosporium Brassicae was described by Berkeley (1), and while his description was incomplete, it unquestionably applies better to a species with long-beaked spores. Saccardo (25) apparently examined a small-spored species, which he recognized as an *Alternaria* and, believing it to be Berkeley's species, made the combination *Alternaria Brassicae*. As a result the name came to be applied erroneously to the small-spored species and this usage is widespread in the literature.

Bolle (3) pointed out this mistake but her treatment of the nomenclature was not in accord with the International Rules. She took the view that since the fungus that Saccardo described was the small-spored species, his combination *A. Brassicae* applied to it. She therefore listed *A. Brassicae* Sacc. among the synonyms of the small-spored species and made a new combination *A. Brassicae* (Berk.) Bolle for the large-spored species. The repercussions of this procedure would be disastrous to the stability of mycological nomenclature. In the first place *A. Brassicae* Bolle is a later homonym of *A. Brassicae* Sacc. and so is invalid under Article 61. Furthermore, if this

procedure were followed, every misdetermination would create a new combination and innumerable new names would be required to avoid creating homonyms as a result of misdeterminations.

In Article 54 of the International Rules it is stated that, "When the specific epithet, on transference to another generic name, has been applied erroneously in its new position to a different plant, the combination must be retained for the plant on which the epithet was originally based." A species can have but one type and any new combinations involving the specific name must be regarded as being based on the type, and not on the fungus studied by the author of the new combination. The specific name must be considered as belonging only to the fungus represented by the type and this cannot be affected by misdeterminations. Therefore Saccardo's combination must be regarded as having been based on Berkeley's type and the correct name for this fungus is *Alternaria Brassicae* (Berk.) Sacc.

Cultures of this fungus (Fig. 6) are whitish with scant aerial mycelium, sometimes marked with concentric zones, and reaching about 3–3.5 cm. in diameter in 10 days. The spores (Figs. 18, 20) are obclavate, long-beaked, light olive-brown, usually with 5 to 12 cross septa and relatively few longitudinal septa, usually borne singly or in short chains of two to three spores, 100–238 × 16–35 μ. The conidiophores are light olive, septate, rarely branched, about 7–11 μ in diameter, very variable in length.

On the seedlings it produces a brownish, water soaked, somewhat sunken lesion. It is less vigorously pathogenic than *A. oleracea* but it will kill the seedlings. Spores are produced more abundantly on the seedling than on the agar.

This fungus has been isolated from the following seeds: *Brassica napus* L. (rape), *B. oleracea* L. var. *capitata* L. (cabbage), *B. oleracea* L. var. *botrytis* L. (cauliflower), *Raphanus sativus* L. (radish). It has been isolated from seeds from Quebec, Ontario, Manitoba, and British Columbia in Canada; Connecticut, Michigan, and California in the United States, and from Holland.

Alternaria Dauci (Kühn) n. comb.

Sporidesmium exitiosum Kühn var. *Dauci* Kühn. Hedw. 1 : 91. 1855.

Alternaria Brassicae (Berk.) var. *Dauci* Lindau. Rab. Kr. Fl. 9 : 260. 1908.

Alternaria Brassicae (Berk.) var. *Dauci* Bolle. Meded. Phytopath. Lab. Willie Commelin Scholten, Baarn, 7 : 42. 1924.

Macrosporium Dauci Rostrup. Tidskr. f. Landøkonomi, 5 R. 7 : 385. 1888.

Macrosporium Carotae Ell. & Langl. Jour. Mycol. 6 : 36. 1890.

This species is the cause of a leaf blight of carrots that may be quite serious at times. It has appeared only rarely on seed. The fungus is close to *A. Brassicae* morphologically but differs in the darker spores with slenderer, more pointed and more branched beaks than in *A. Brassicae*, in cultural characters, and host relationships.

The earliest name for this species appears to be that proposed by Kühn (11) when he described it as *Sporidesmium exitiosum* var. *Dauci*. According to Article 58 of the International Rules when a variety is raised to a species the name first used in that rank is valid. Rostrup (24) raised Kühn's variety to specific rank as *Macrosporium Dauci*.

Neergaard (19) has shown from an examination of Rostrup's slides that his fungus was misidentified. He did not have Kühn's fungus but the species that was later described as *A. radicina*. Nevertheless here, as in *A. Brassicae*, Article 54 applies, and Rostrup's combination must be considered as having been based on Kühn's type.

There seems to be little doubt that the fungus described by Ellis and Langlois (8) as *Macrosporium Carotae* is the same species.

Cultures (Fig. 7) on malt agar are rather slow growing reaching 3–5 cm. in diameter in 10 days. They vary from dull ochre yellow to olive or sometimes to grayish white depending on the amount of aerial mycelium, which varies from velvety to fluffy cottony. There is a pronounced yellowish-orange to reddish chromogenesis in the agar. The conidiophores are olive-brown, septate, simple or branched, variable in length, about 6–10 μ in diameter. The conidia (Figs. 15, 21) are obclavate, with long attenuated, frequently branched beaks, dark olive-brown, with 7 to 10 transverse septa and relatively few longitudinal septa, borne singly or in short chains of two to three spores, (100)–150–250–(310) × 15–25 μ.

It has been isolated from seeds of *Daucus carota* L. var. *sativa* DC. from Nova Scotia and Ontario.

Alternaria linicola sp. nov.

Coloniis effusis, gossypinis, leviter zonatis, olivaceo-cinereis; hyphis septatis, ramosis, hyalinis vel pallide fusco-olivaceis, (2)–4–7 μ diam.; conidiophoris pallide olivaceo-brunneis, septatis, simplicibus, 5–8 μ diam.; conidiis, solitariis, obclavatis, longe rostratis, levibus, olivaceo-brunneis, 7–11 transverse septatis, muriformibus ad septa leviter constrictis, (130)–150–300 × (15)–17–24 μ. Hab. in seminibus *Lini usitatissimi* L.

Colonies on malt agar (Fig. 8) effused, cottony, slightly concentrically zoned, reaching about 6–7 cm. in diameter at 10 days, "deep olive-gray" to "dark olive-gray", paler at the centre to "pale olive-gray" (Ridgway), hyphae septate, branched, hyaline to pale smoky olive, (2)–4–7 μ in diameter, conidiophores pale olive-brown, septate, simple, 5–8 μ in diameter, very variable in length; conidia (Figs. 16, 22) not observed in chains, obclavate, gradually attenuated above into a long, slender, sometimes branched beak, olive-brown, smooth, muriform, with 7 to 11 transverse septa, slightly constricted at the septa, (130)–150–300 × (15)–17–24 μ.

Host: in seeds of *Linum usitatissimum* L.

Type: Herb. Div. Botany and Plant Pathology, Dept. of Agr. Ottawa. 14083.

Distribution: This species has been isolated from seeds originating in Quebec, Ontario, Manitoba, Saskatchewan, and Alberta.

It is difficult to distinguish cultures of *A. linicola* from non-sporulating strains of *A. tenuis* except by the effect on the seedlings (Figs. 8, 2), although cultures of *A. linicola* are usually more grayish in colour than those of *A. tenuis*. Seedlings from seed infected with *A. linicola* are usually killed in the 10 day period before readings are taken and any other seedlings in the plate that may chance to come in contact with the colony are vigorously attacked. The infected tissues become soft and a tuft of grayish-white, cottony aerial mycelium arises from the seedling. Infected seedlings develop a characteristic pinkish colour very similar to that observed in seedlings attacked by *Colletotrichum Lini* (West.) Toch. This similarity was so marked that it was at first thought that the effect noted was caused by a mixture of *C. Lini* and *A. tenuis*. This conclusion appeared to be supported by the fact that conidia of *A. tenuis* were sometimes found on these seedlings. However it soon became evident that this was due to a mixture of *A. tenuis* with a pathogenic fungus other than *C. Lini* for mixtures of *A. tenuis* and *C. Lini* could invariably be detected by the presence of conidia of *C. Lini*.

Cultures of the pathogenic species failed to produce spores on agar but when inoculations were made on flax plants in the greenhouse, infection was readily secured and large *Alternaria* conidia of the "*Brassicae*" type were produced. Six single conidia were isolated and each returned the typical culture. It has since been found possible to obtain conidia on agar using the mutilation method described by Rands (22).

Since no description of any *Alternaria* on flax appeared to fit this species it has been necessary to describe it as new. *A. Lini* described by Dey (6) is evidently an *Alternaria* of the *tenuis* type. *A. linicola* is close to *A. Dauci* in morphology but differs in cultural characters and host relationships.

We have no information concerning the effect of *A. linicola* in the field, or the conditions required for infection under field conditions. It would seem probable from the virulence of its attack on seedlings in the plates, and from the ease with which infection was obtained in the greenhouse, that under suitable conditions this fungus would be capable of causing considerable damage, probably as a leaf blight and in pre-emergence mortality.

Alternaria radicina Meier, Drechsler, & Eddy. Phytopath. 12 : 164. 1922.

Stemphylium radicinum Neerg. Aarsberetn. fra J. E. Ohlsens Enkes Plantepatol. Lab., 1 April 1938–31 Marts 1939: 14. 1939.

This species was first described by Meier, Drechsler, and Eddy (16) and designated as the cause of black rot of carrots, a storage decay. Since then it has become increasingly recognized as the cause of serious losses in carrots. Neergaard (19) reported that it could cause a root rot, and damping-off of seedlings of celery. He also isolated it from parsnip and parsley where it did not appear to be causing much damage. From seeds we have isolated it

frequently from carrot, occasionally from parsley, and once from vegetable marrow.

On malt agar the cultures (Fig. 3) produce fairly abundant aerial mycelium, which varies from gray to olive-gray or usually nearly black, whitish at the margin. The spores are borne terminally and usually singly on simple or branched, septate, olive-brown to dark brown conidiophores, which are 4–6 μ in diameter but variable in length. The conidia (Figs. 14, 23) are dark brown, obovoid to obclavate or ellipsoid, usually with three to seven transverse septa and several longitudinal septa, more or less constricted at the septa, (25)–30–60–(73) × (15)–20–27–(35) μ.

It has been isolated from seeds of *Cucurbita Pepo* L. (vegetable marrow), *Daucus carota* L. var. *sativa* DC. (carrot), *Petroselinum hortense* Hoffm. (parsley), received from Nova Scotia, Manitoba, British Columbia, Connecticut, Minnesota, and California.

The generic position of this fungus is to some extent in doubt. Neergaard (20) referred it to *Stemphylium* and there is no doubt that it exhibits some of the characters of this genus. *Stemphylium* is distinguished from *Alternaria* by having the spores borne singly rather than in chains and this distinction is correlated with the difference in spore shape. In *Stemphylium* both ends of the spore are rounded whereas in *Alternaria* the spore tapers more or less to the distal end.

When spores from near the growing margin of a colony were examined, a considerable number were observed that tapered toward the distal end as in *Alternaria* but when mounts were made from older cultures few of these spores were found. The spores appeared to undergo secondary thickening in such a way that they became more ellipsoid than clavate, and rounded at both ends. They thus resembled *Stemphylium* spores in shape although they lacked the conspicuous constriction at the median septum that is apparently characteristic of *Stemphylium*.

Meier, Drechsler, and Eddy (16) stated that chains were produced only on media poor in organic nutrient. We failed to find chains in cultures on malt agar and also on tap water agar. On the latter medium instances were observed of chain-like development in which the conidiophore had appeared to undergo basipetal fragmentation, such as was noted by Groves and Skolko (9) in *Stemphylium botryosum*, so that the distal spore was the oldest. This observation would provide additional evidence of the relationship of this fungus to *Stemphylium*. However, on the basis of Drechsler's figure, which clearly illustrates short chains in which the distal spore is the younger, we are retaining this species in *Alternaria*.

Alternaria oleracea Milbr. Botan. Gaz. 74 : 321. 1922.

In discussing *A. Brassicae* above, it was noted that two diseases, of which *Alternaria* species were the cause, had been described on cruciferous plants. The diseases have been well described by Weimer (27, 28). It is now clear

that the gray leaf spot is caused by *A. Brassicae*, which Weimer (28) treated under the name *A. herculea*, a later synonym. Since the work of Bolle (3) the black leaf spot has been generally attributed to *A. circinans* (B. & C.) Bolle but it now appears doubtful whether there are not two fungi involved in this disease. Weimer (27) identified the species that he found to be the cause of black leaf spot as *A. Brassicae* but from his descriptions and figures he certainly had the fungus that we are calling *A. oleracea*.

A. oleracea, which was described by Milbrath (17), was listed by Bolle (3) as a synonym of *A. circinans*, but, as indicated by the interrogation mark placed in front of *A. oleracea*, she evidently had some doubt concerning their identity. Since it appeared that if these two names were actually synonyms, *A. oleracea* was the valid name under Article 58 of the International Rules, and it seemed desirable to check the identity of the fungi more closely. Accordingly specimens were sent to Dr. D. H. Linder with the request that he compare them with the cotype material of *A. circinans* in the Curtis Herbarium[1].

Dr. Linder replied in part as follows: "The spores of your specimen are heavy walled and dark in comparison with those of *A. circinans*, also mostly smaller and fewer septate, whereas those of the latter species tend to be many-septate, cylindrical, and arcuate. It is interesting to note that the few-septate spores of your specimen tend to have somewhat swollen basal cells and the 1 septate spores are pyriform. In *A. circinans* whenever the basal cells are swollen it is usually in the many-septate spores and usually when one or more of the basal cells is or are septate. I think *A. circinans* should be kept distinct for the long, many-septate form and *A. oleracea* for the forms that resemble your culture." Dr. Milbrath in a letter also expressed doubt that *A. circinans* and *A. oleracea* were synonyms.

Therefore in the face of the doubt expressed by both Bolle and Milbrath regarding the identity of the two species, and Linder's finding that typical *A. oleracea* material did not agree with the cotype of *A. circinans*, we have no alternative but to adopt the name *A. oleracea* for our fungus. The fact that only one disease has been recognized might be regarded as evidence for the identity of the two species, especially since it is well known that spores of *Alternaria* species may undergo considerable variation in size and shape under cultural conditions. However even if they should eventually prove to be synonymous, *A. oleracea* would still be the valid name since the combination, *Macrosporium circinans* B. & C., scarcely meets the requirements of effective publication and all other earlier names cited by Bolle are only of varietal rank.

We have isolated this fungus only from seeds of cruciferous plants with the exception of one isolation from scarlet runner beans. The cultures (Fig. 5) are dark greenish olive to olive-brown with copious spore production and scant development of aerial mycelium. The conidiophores are olive-

[1] *Dr. Linder states that Bolle's citation of the Curtis number is a typographical error. It should be 2461 not 2411.*

brown, septate, simple or branched, 4–6 μ in diameter, variable in length. The conidia (Figs. 13, 24) are obclavate, not beaked, olive-brown, smooth, with 3–7–(10) transverse septa and relatively few longitudinal septa, constricted at the septa, (20)–30–50–(80) $\times$ 10–15–(18) μ.

This species has been isolated from the following seeds: *Brassica napobrassica* Milb. (swedes), *B. oleracea* L. var. *botrytis* L. (broccoli, cauliflower), *B. oleracea* L. var. *capitata* L. (cabbage), *Phaseolus coccineus* L. (scarlet runner beans), *Raphanus sativus* L. (radish).

The species is evidently of wide geographic distribution. We have isolated it from seeds from Prince Edward Island, Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, and British Columbia, in Canada; from New Jersey, Connecticut, Pennsylvania, Michigan, Minneapolis, and Ohio in the United States, and from Denmark and Holland.

Under plating conditions *A. oleracea* is strongly pathogenic. Infected seedlings are soon killed and after 10 days are usually completely covered with a profuse growth of conidiophores and conidia and the tissues are soft and rotted. It is of interest to note that in the single instance in which this fungus was isolated from scarlet runner beans, the seedling from the seed that gave rise to the colony appeared to be quite normal and was not attacked by the fungus. Transfers from this colony were made to plates on which were growing healthy, 10-day-old seedlings of cabbage and radish and both of these were vigorously attacked.

A number of tests have been conducted on Swede seeds by placing them on moist blotters without surface sterilization and keeping them in a Jacobsen germinator at room temperature for 10 days. Under these conditions *A. tenuis* appears on practically 100% of the seedlings, forming a whitish weft of mycelium around the base and producing the characteristic spores. However it does not appear to cause any injury whatever to the seedlings and by about 17 to 18 days it has disappeared. On the other hand, *A. oleracea*, if present, attacks the seedlings vigorously (Fig. 10), causing a brownish, sunken, water-soaked lesion at the base of the hypocotyl and advancing upwards, causing the plant to fall over and die. The hypocotyl is sharply constricted at the infected area and there is a profuse development of conidiophores and conidia. There is evidence of differences in varietal susceptibility to the pathogen.

At the present time we have no information as to the effect of *A. oleracea* on seedlings in the field, or of conditions required to produce infection. It would seem probable that under suitable conditions this fungus would be capable of causing considerable damage.

Alternaria Raphani sp. nov.

Coloniis effusis, gossypinis, pallide cinereis; hyphis septatis, ramosis, hyalinis vel fusco-olivaceis, 2–5–(7) μ diam.; chlamydosporis numerosis, olivaceo-brunneis, primo unicellularis, orbicularibus, dein multicellularis,

irregularibusque; conidiophoris olivaceo-brunneis, septatis, simplicibus vel interdum ramosis 4–7 μ diam.; conidiis catenulatis, obclavatis, subrostratis, levibus, olivaceo-brunneis, 3–9 transverse septatis, muriformibus, ad septa constrictis, (40)–50–70–(94) × 15–25–(45) μ in cultura, (55)–70–115–(135) × 14–18–(25) μ in folliis inoculatis; maculis in siliquis, suborbicularibus, nigris, usque 4 mm. diam.

Hab. in siliquis et seminibus *Raphani sativi* L.

Colonies on malt agar effused, cottony, not zoned, reaching 6–8 cm. in diameter in 10 days, "pale olive-gray" to "pale gull gray", occasionally darker to "olive-gray" or "iron gray" (Ridgway), hyphae septate, branched, hyaline to smoky olive, 2–5–(7) μ in diameter; chlamydospores numerous, olive-brown, at first one-celled, round, finally many-celled and irregular in shape; conidiophores olive-brown, septate, simple or sometimes branched, very variable in length, 4–7 μ in diameter, usually enlarged toward tip; conidia (Figs. 17, 25) catenulate in short chains, obclavate, beaked but the beaks usually short, smooth, olive-brown, muriform, with 3 to 9 transverse septa and numerous longitudinal septa, constricted at the septa, (40)–50–70–(94) × 15–25–(45) μ on seedlings on agar in Petri dishes, (55)–70–115–(135) × 14–18–(25) μ on inoculated leaves in the greenhouse; spots on seed pods (Fig. 11), roughly circular, black, up to 4 mm. in diameter.

Host: in seeds and seed pods of *Raphanus sativus* L.

Type: Herb. Div. Botany and Plant Pathology, Dept. of Agr. Ottawa. 13624.

Distribution: This fungus has been isolated from radish seeds originating in Ontario, Quebec, and British Columbia and from imported seeds from California, Michigan, Minnesota, New Jersey, Ohio, and Pennsylvania.

In addition to *A. Brassicae* and *A. oleracea*, which are pathogenic to seedlings of various cruciferous hosts, another species has been found occurring, so far, only on radish seeds, and extremely pathogenic to the seedlings. We have also obtained evidence that it causes a spotting of the seed pods in the field. We at first thought this species might be *A. tenuissima* (Fr.) Wilts., and specimens were sent to Dr. S. P. Wiltshire for confirmation. In a letter Dr. Wiltshire stated that it is definitely not this species, but is identical with the *Alternaria* species reported by Ware on *Matthiola*[1] and also with the species on *Brassica chinensis* reported by Yoshii as *Alternaria Brassicae* (Berk.) Sacc. var. *macrospora* Sacc.[2] It has not been possible to identify this fungus with any previously described *Alternaria* and it is, therefore, proposed as a new species.

[1] *Ware, W. M. Garden Chron. 100 : 236-237. 1936.*
[2] *Yoshii, H. Bull. Sci. Fak. Ferkult. Kjusu Imp. Univ. 5 : 221-235. 1933.*

Colonies from infected seeds (Fig. 9) plated on malt agar after 10 days somewhat resemble colonies of *A. tenuis* but can be distinguished by their paler colour, the presence of chlamydospores, and by the effect on the seedlings. The aerial mycelium is grayish to nearly white and does not exhibit the greenish tints of *A. tenuis*. Characteristic cell formations, here referred to as chlamydospores, are usually produced in abundance on the vegetative mycelium. They arise first as single, thick-walled, globose intercalary cells, developing into agglomerations of many large, dark, thick-walled cells, and are very variable in size and shape (Fig. 25B). They appear to resemble the "sclerotium-like formations" described by Neergaard (19) as occurring in old, contaminated cultures of *A. radicina*. However in *A. Raphani* they appear in young cultures that show no evidence of contamination and also in cultures originating from single spores.

Seedlings from infected seeds are usually killed before they have developed very far and there is a profuse development of aerial mycelium on the dead seedling, forming a cottony tuft (Fig. 9). Spores are only rarely produced on the agar but can usually be found on the seedling if the cottony tuft of aerial mycelium is removed.

Inoculations were made on seedlings of radish, cauliflower, broccoli, swedes, cabbage, and wallflower (*Cheiranthus Cheiri* L.) in the greenhouse by putting bits of agar and mycelium on the leaves and placing bell jars over the plants. Inoculations were successful on all but the wallflower. The latter was included on the possibility that the fungus might be *Alternaria Cheiranthi* (Lib. ex. Fr.) Bolle, but its failure to infect *Cheiranthus* would appear to eliminate this species. Infection on cabbage was weak but the fungus appeared to attack the other plants inoculated with about equal severity.

Spores were slightly different from the spores found in plate cultures. As observed on the host the spores were mostly longer and narrower than those found in the plate cultures (Fig. 25A). The differences are noted in the description above and they should probably be considered as variations due to the substrata, because five single spores of the long narrow type isolated from a lesion on an inoculated radish seedling in the greenhouse each yielded a culture typical of that found on the plates.

On several occasions, specimens of radish seed pods bearing black circular spots have been received by the Division of Botany and Plant Pathology. No spores were found on these spots but isolations from the tissue yielded cultures of *A. Raphani*.

The results of the examination of seed from five different crops from the same seed stock of radish var. Scarlet Globe are given below. These crops were grown in widely separated localities within a radius of 100 miles from Ottawa, and all showed the black spots on the seed pods believed to be caused by *A. Raphani*.

Locality	Acreage	Yield in lb.	Official germination, %	Fungi, %	
Martintown, Ont.	½	35	14	*A. Raphani*	22
				A. tenuis	40
				Fusarium spp.	18
				Total fungi	80
				Bacteria	6
Kinburn, Ont.	3½	200	18	*A. Raphani*	72
				A. tenuis	35
				Fusarium spp.	6
				Chaetomium sp.	1
				Cunninghamella echinulata	1
				Gonatobotrys simplex	1
				Cladosporium herbarum	2
				Nigrospora sphaerica	1
				Mucor spp.	2
				Penicillium spp.	2
				Rhizopus sp.	1
				Total fungi	124
				Bacteria	7
Richmond, Ont.	8	820	40	*A. Raphani*	58
				A. tenuis	38
				A. Brassicae	2
				Total fungi	98
Renfrew, Ont.	1	350	50	*A. Raphani*	43
				A. tenuis	25
				A. Brassicae	2
				Fusarium sp.	1
				Trichoderma viride	1
				Total fungi	72
				Bacteria	7
Embrun, Ont.	1	10	65	*A. Raphani*	19
				A. tenuis	31
				A. Brassicae	1
				Fusarium spp.	2
				Chaetomium spp.	5
				Total fungi	58
				Bacteria	35

It will be noted that in all but the Martintown sample there is a correlation between the germination of the seed and the percentage of *A. Raphani*, and also that *A. Raphani* is the only pathogenic fungus appearing consistently in all the samples in a high percentage. The discrepancy in the Martintown sample could be accounted for by the high percentage of *Fusarium* present in this sample.

There does not appear to be a definite correlation between the percentage of *A. Raphani* and the yield, but too many other factors may affect the yield to expect a very close correlation in this respect. It will be noted that the yields are extremely low.

An attempt was made to obtain a sample of the original seed but the only available one had been treated with red copper oxide so that the results are not strictly comparable. When this seed was plated, *A. Raphani* was isolated from 4% of the seeds, indicating at least that the fungus was definitely present in the original seed.

Seed from the Richmond sample was treated with four common dusts and plated again. A slight reduction in the percentage of *A. Raphani* was obtained as follows: untreated, 58%; Ceresan, 42%; Semesan, 46%; Arasan, 46%; Spergon, 38%. On this slight evidence it would seem that seed treatment is of no value and that the only satisfactory control measure is by the use of sound seed.

Discussion

The difficulties of identifying species in the genus *Alternaria* have been recognized by everyone who has studied the group and in our present state of knowledge, anything like a comprehensive treatment of the genus is impossible. Until the life histories are more completely known, and a careful comparative study of a large number of species has been made it is only possible to suggest specific limitations along what appear to be convenient and workable lines.

Although the unnecessary multiplication of species of fungi is to be avoided, and we are in general agreement with the views expressed by Bisby (2) regarding the necessity for synthesis of many fungus species, nevertheless it is a recognized principle of science that analysis must precede synthesis. Thus in groups of fungi in which the life histories are not completely known, and in which the range of variation is not clear, the taxonomic value of the characters observed is uncertain. It would seem necessary in preliminary studies to separate species on any recognizable morphological differences or other characters that are convenient to use, such as the host relationship. Subsequent accumulation of knowledge of the group will make it possible to decide whether these species can be maintained as separate entities or synthesized into a broader concept.

This is, in general, what has occurred in the development of our knowledge of the fungi, but there are many groups, of which *Alternaria* is one, in which we are still in the analytical stage. It is felt that at this stage it is preferable to lay emphasis on the analytical aspects and to err, perhaps, in the direction of the splitter. If as a result of subsequent investigation the differences are found to be inconstant or otherwise of no significance taxonomically, it causes comparatively little confusion to place the names in synonymy. On the other hand, when different species are confused under the same name, either as a

result of undue conservatism in describing species, or of premature synthesis of existing species, or simply because of misdeterminations, it usually is a much more difficult task to disentangle them, and frequently much confusion ensues, especially when pathogenic species are concerned.

These considerations are offered in explanation of what may seem to be inconsistency in the treatment of the *Alternaria* species discussed in this paper. We have, for example, united under *A. tenuis*, strains that exhibit considerably more morphological differences than can be found between *A. Dauci* and *A. linicola*, the latter of which we have proposed as a new species.

The variations in the strains grouped under *A. tenuis* have been referred to above under the discussion of this species. In the beginning of the work on seed-borne fungi an attempt was made to distinguish different species among these strains. Whereas the range in spore size is given as 20–50 × 10–16 μ, in some strains the spores are predominatingly toward the upper end of the range, while in others they are predominatingly toward the lower. Some strains have rough spores, while others have smooth spores and in some the spores are pale olive-brown, in others dark brown. These and other characters were used as a basis for separating strains but as more cultures accumulated it became evident that no correlation existed between the different characters and that they did not always remain constant in subsequent transfers. The fact that the variations observed did not seem to be in any way correlated with the host also appeared to be significant. It was finally concluded that the only workable method of handling these forms was to group them all together and consider them as strains or morphotypes of one species. This concept will result in the synthesis of a great many *Alternaria* species including *A. fasciculata* (E. & M.) Jones & Grout, *A. humicola* Oud., *A. maritima* Sutherland, and many other species that have been described chiefly on the basis of the host.

Most of the strains of *A. tenuis* appear to be non-pathogenic but, as noted above, pathogenic species that would fall within the concept of this species have been described. It is suggested that unless distinctive characters other than pathogenicity can be demonstrated in these strains, that they might well be regarded as of varietal rather than specific rank.

The other group of species, which includes *A. Brassicae*, *A. Dauci*, and *A. linicola*, is characterized by the large spores with long attenuated beaks, usually borne singly or in short chains of two or three spores, and by the generally parasitic habit. We are of the opinion that although it may eventually become possible to consider these and related forms as varieties of one species, at the present time it is not desirable to synthesize this group.

From the seed investigations it is evident so far that a correlation exists between cultural characters and host. For example all the cultures of a large spored *Alternaria* isolated from seeds of cruciferous plants have had a characteristic appearance by which they could be readily recognized once one became familiar with them. The variation between isolates of *A. Brassicae* from the

same or different cruciferous hosts is less than the variation between strains of *A. tenuis* from any one host. Likewise cultures of *A. linicola* are quite uniform in appearance but very different from either *A. Brassicae* or *A. Dauci*.

In connection with *A. Dauci*, however, it should be mentioned that cultures of this species were practically indistinguishable from cultures of *A. Solani* (Ell. & Mart.) Jones & Grout received from Dr. William Newton, Saanichton, B.C., and isolated from lesions on potato leaves. *A. Solani* is another member of this large-spored group that we have not isolated from seeds, although it has been reported by Massee (15), Miller and Crosier (18), and Neergaard (21) as being seed-borne in tomatoes. It is evidently very close to *A. Dauci* both in cultural characters and morphology of spores, and apart from the host, there seems to be little basis for separation of these two species.

It is thus evident that a great need exists for much further investigation of these species, including comparative cultural studies of a large number of strains, cross inoculations, and the search for perfect stages. Possibly from such studies a better conception of the host relationships and specific limitations will emerge. In the meantime from a pathological standpoint it is desirable to keep them distinct and a separation based on the host supported by the differences observed in the cultural characters would seem to provide the most practical and workable method of handling these parasitic species.

It is, therefore, hoped that the inclusion of the small-spored forms under *A. tenuis* on the one hand, and the separation of the large-spored forms into species on the other hand, will be looked upon as not inherently inconsistent, but rather as an attempt to place these forms on a workable basis according to the stage of development of our knowledge concerning them. The remaining three species, *A. oleracea*, *A. Raphani*, and *A. radicina* seem to be sufficiently clear cut that their status as species need not be questioned.

Acknowledgments

We are indebted to Dr. D. H. Linder, for his assistance in the identification of *A. oleracea* and for comparing our material with the type of *A. circinans*; and to Dr. S. P. Wiltshire and Mr. E. W. Mason for examining specimens and cultures of *A. Raphani* in order to eliminate the possibility of it being identical with *A. tenuissima* (Fr.) Wilts.

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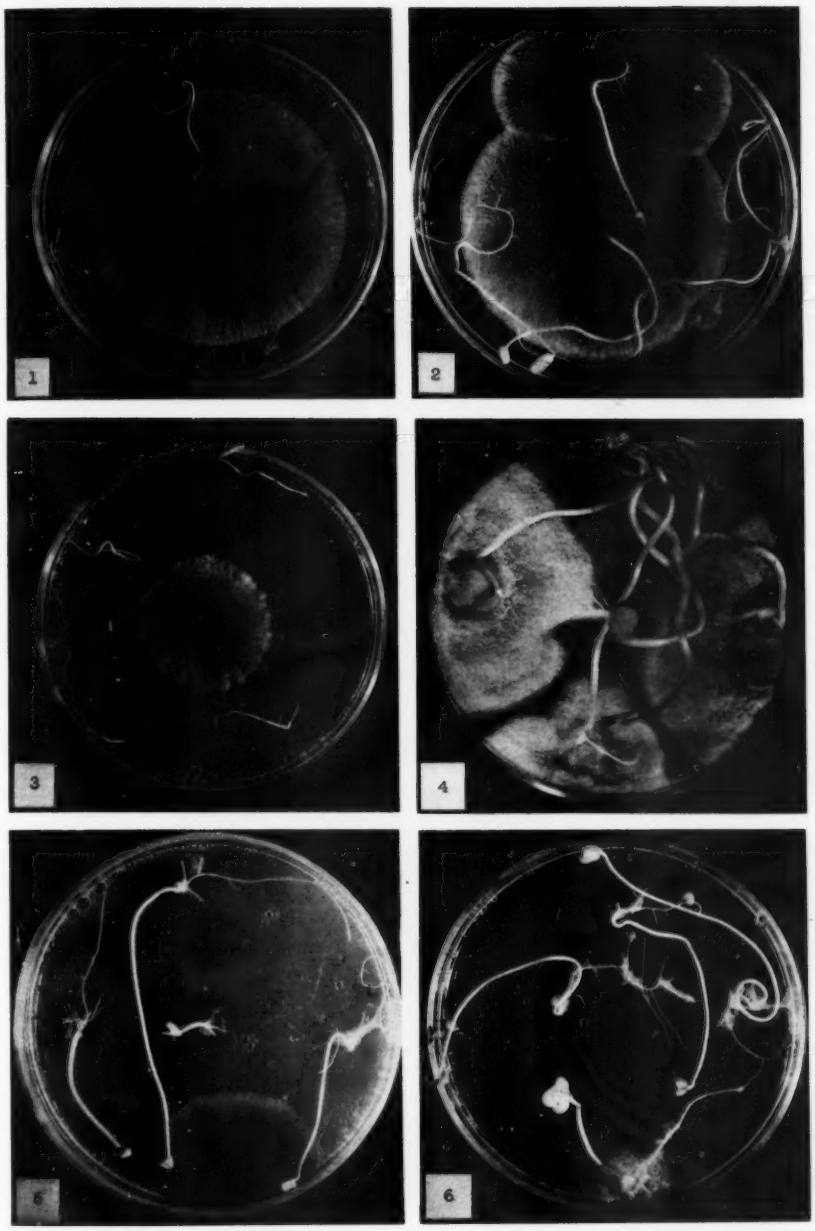
PLATE 1



FIGS. 1 TO 6. *Cultures of Alternaria species.* 1. *A. tenuis on carrot seeds on malt agar at 10 days;* 2. *A. tenuis on flax seeds on malt at 10 days;* 3. *A. radicina on carrot seeds on malt agar at 10 days;* 4. *A. tenuis on pea seeds on pea agar at 10 days;* 5. *A. oleracea on cabbage seeds on malt agar at 10 days;* 6. *A. Brassicae on radish seeds on malt agar at 10 days.*

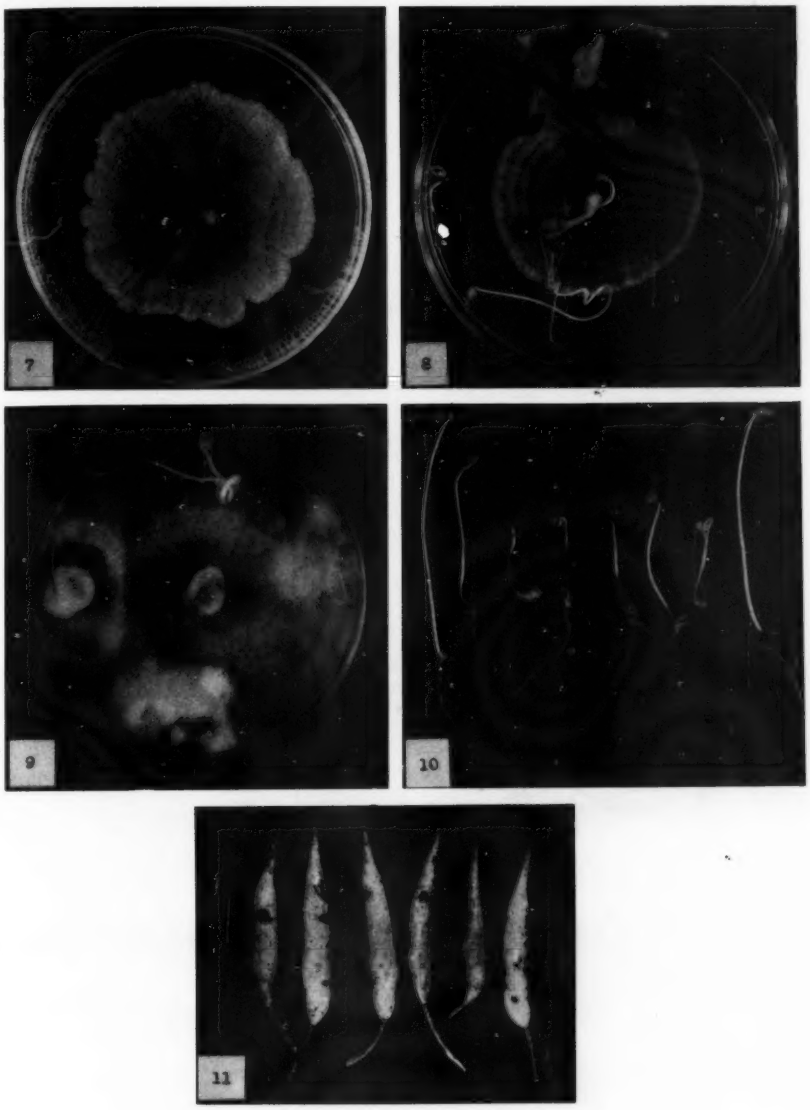
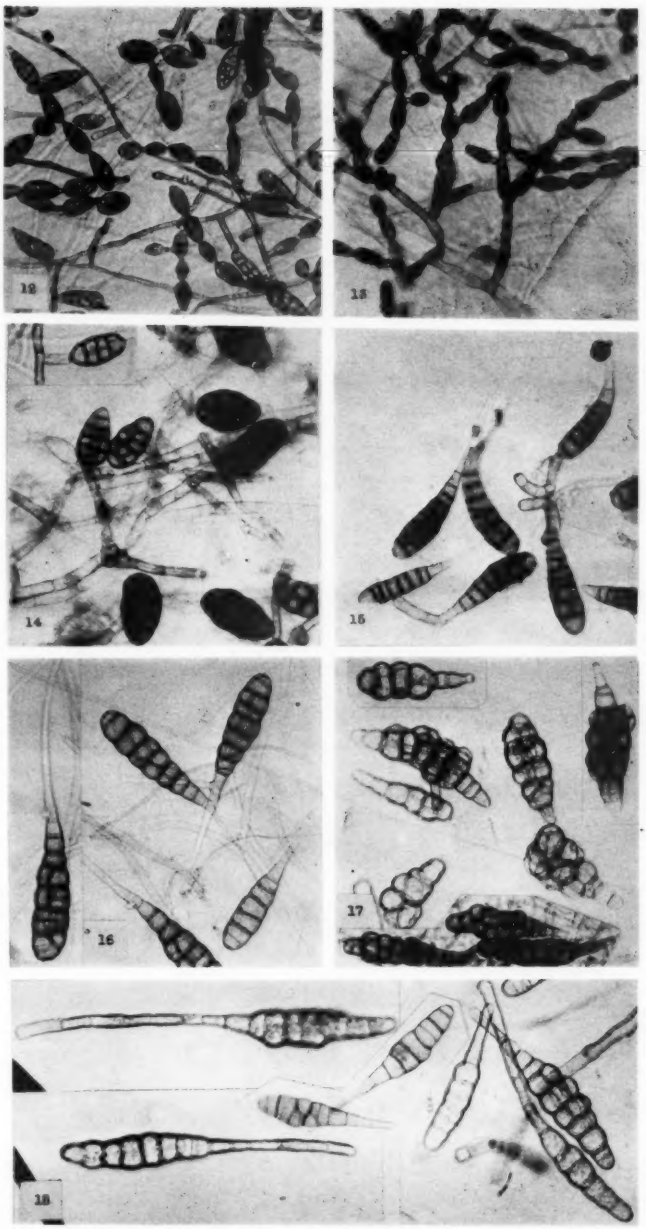


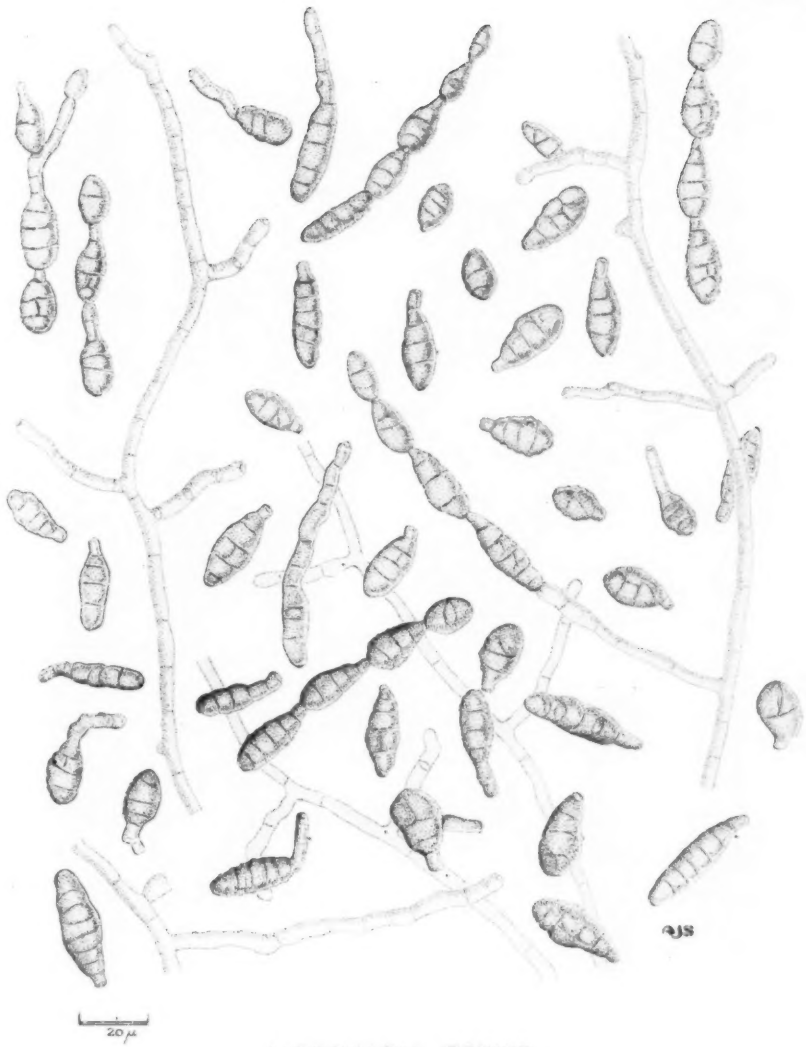
FIG. 7. *A. Dauci culture from carrot seeds plated on malt agar at 12 days.*
FIG. 8. *A. linicola on flax seeds on malt agar at 10 days.*
FIG. 9. *A. Raphani on radish seeds on malt agar at 10 days.*
FIG. 10. *A. oleracea on Swede seedlings from blotters, six diseased and two healthy.*
FIG. 11. *A. Raphani on radish seed pods showing lesions.*

PLATE III



FIGS. 12 TO 18. *Photographs of spores of Alternaria species.* 12. *A. tenuis;* 13. *A. oleracea;* 14. *A. radicina;* 15. *A. Dauci;* 16. *A. linicola;* 17. *A. Raphani;* 18. *A. Brassicae.*

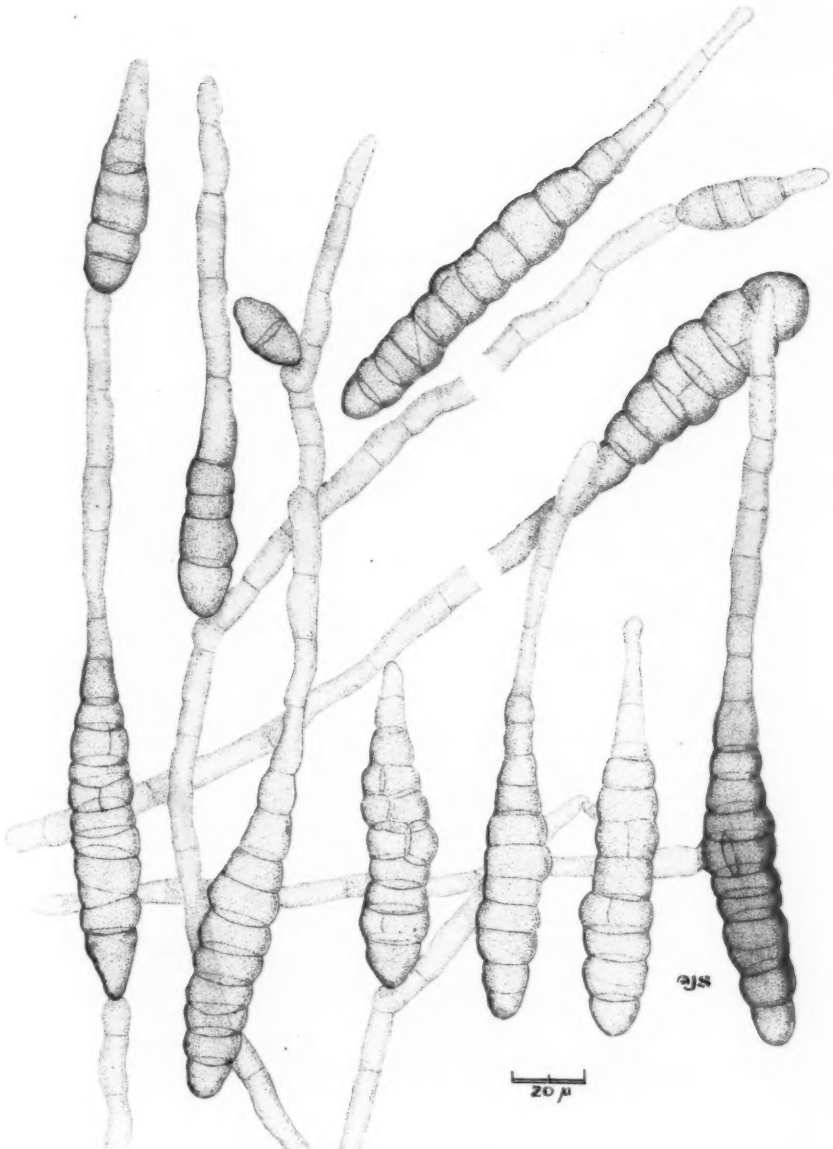
PLATE IV



ALTERNARIA TENUIS AUCT.

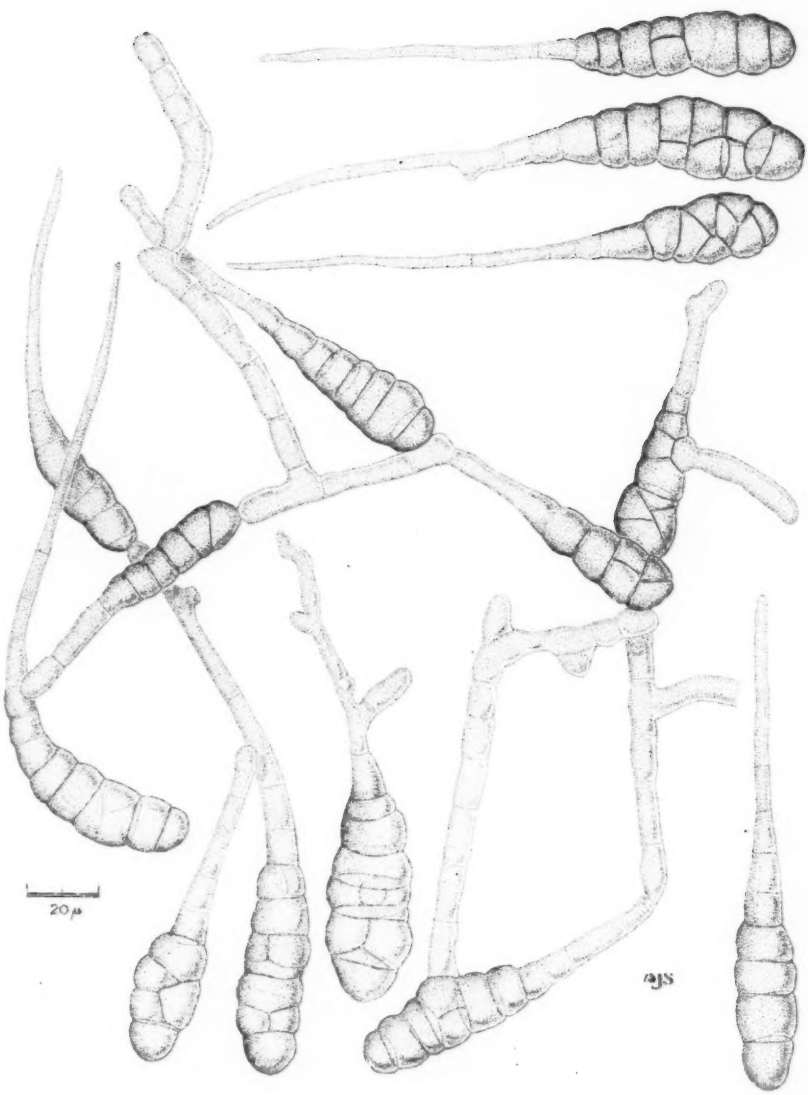
FIG. 19

PLATE V



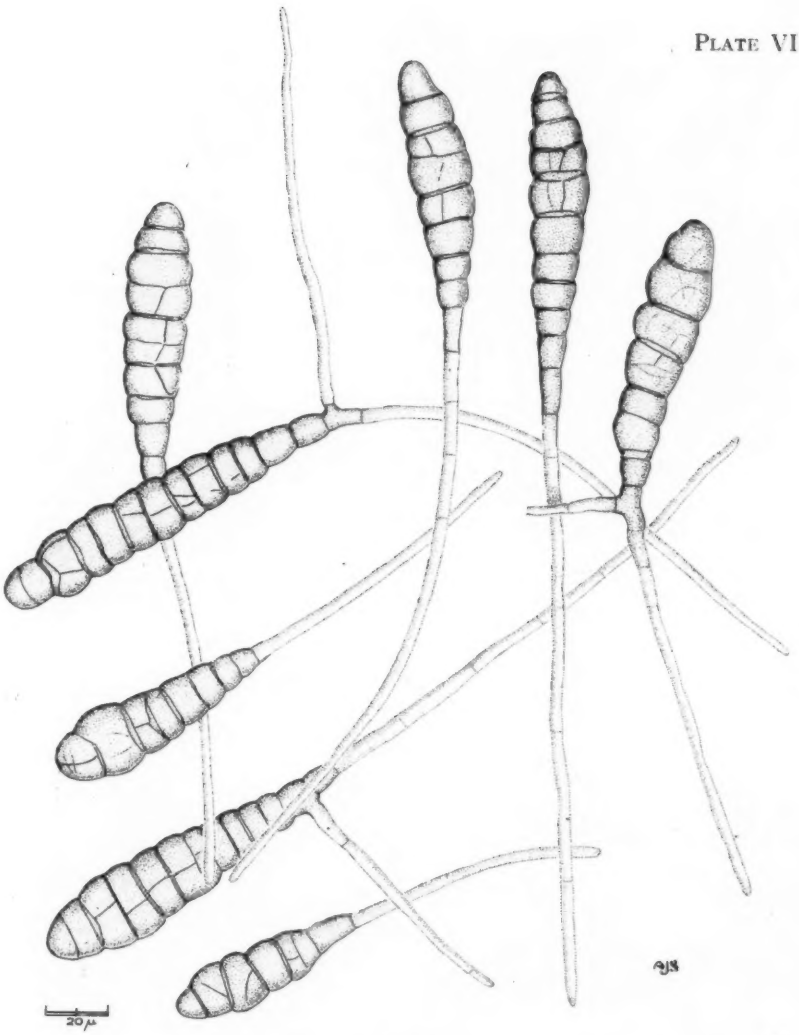
ALTERNARIA BRASSICAE (BERK.) SACC.

FIG. 20



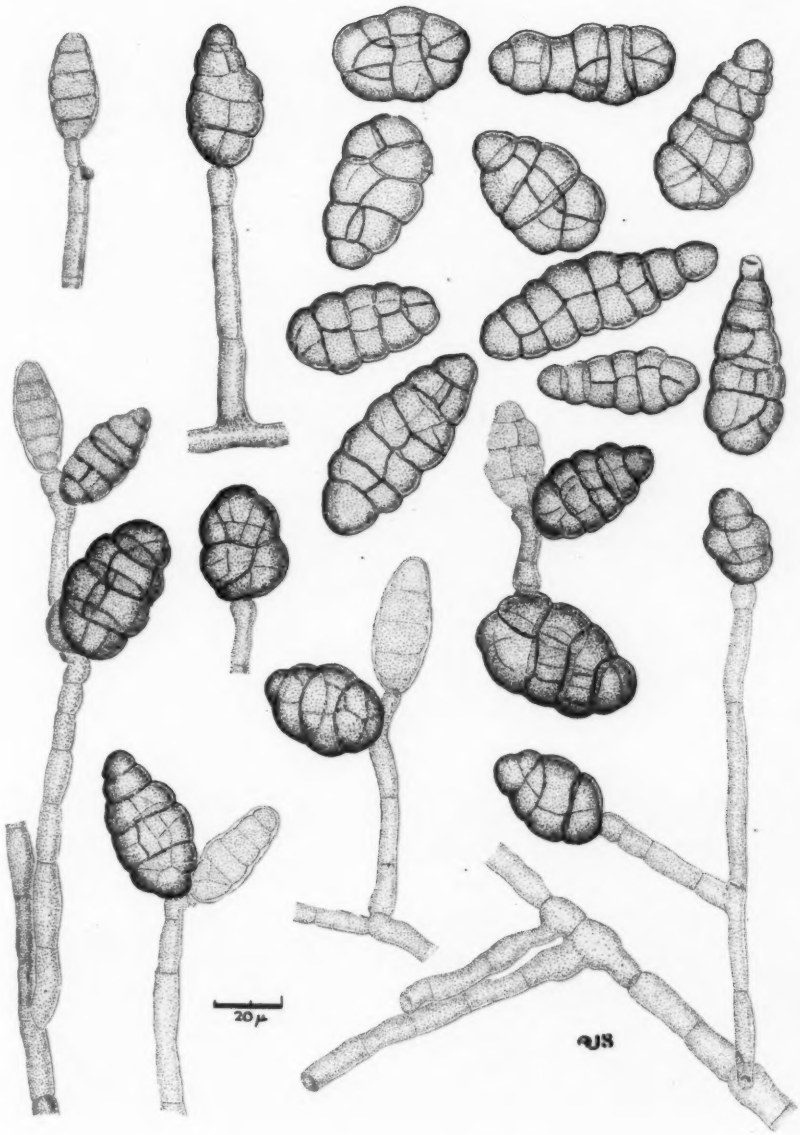
ALTERNARIA DAUCI (KÜHN)

FIG. 21



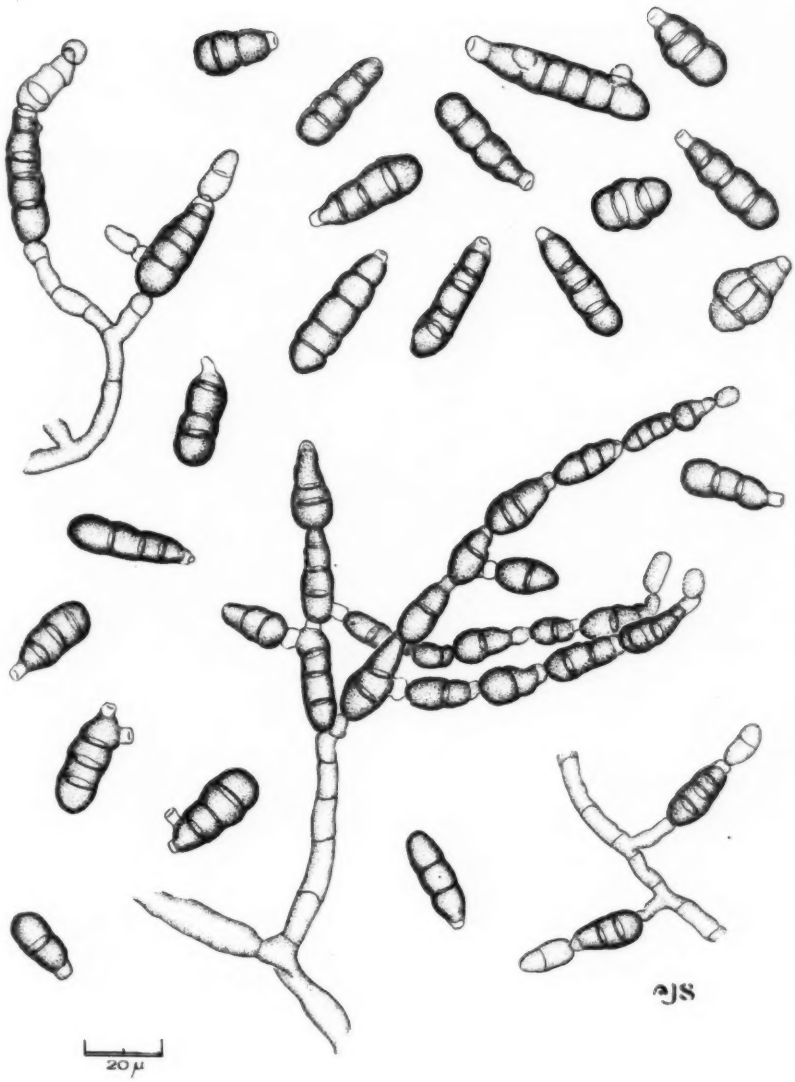
ALTERNARIA LINICOLA N. SP.

FIG. 22



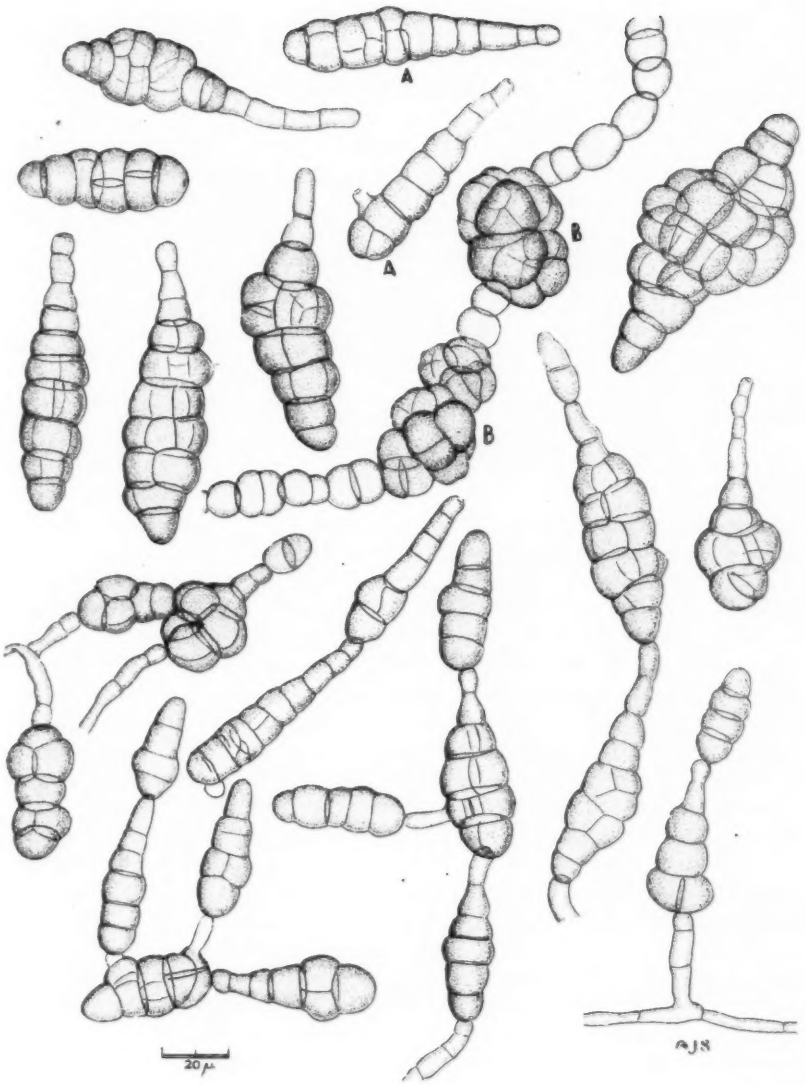
ALTERNARIA RADICINA MEIER, DRECHSLER, AND EDDY

FIG. 23



ALTERNARIA OLERACEA MILBR.

FIG. 24



ALTERNARIA RAPHANI N. SP.

FIG. 25

STUDIES WITH *BACILLUS POLYMYXA*

I. SOME FACTORS AFFECTING THE FERMENTATION OF WHEAT BY *BACILLUS POLYMYXA*[1]

By H. Katznelson[2]

Abstract

Factors affecting the fermentation of 15% wheat mashes by strains of *Bacillus polymyxa*, under laboratory conditions, were studied with a view of obtaining maximum yields of 2,3 butanediol, a compound that can be converted to butadiene, an essential precursor of synthetic rubber. Neither the nature of the inoculating medium nor the age of the inoculum affected the fermentation but small amounts (0.5%) of the inoculum lowered the yield. Increasing the surface of the fermentation mixture resulted in improved yields of the diol as did the addition of yeast extract. Different strains varied markedly in fermentative ability; of 82 strains tested, 10 gave excellent results (over 3% diol). Combinations of two strains (phage-susceptible and phage-resistant) also gave satisfactory yields but mixtures of 5 or 10 caused a sharp drop in diol production. Low-grade, low-quality wheat samples were as satisfactory as samples of high-grade wheat in diol yield capacity. Elevator screenings were capable of being fermented by *A. polymyxa* although low yields were obtained, owing, undoubtedly, to the low starch content of these materials. However, since screenings command a low price on the market, it may be economically practical to consider their utilization for butanediol production.

Recent intensive studies on the commercial production of 2,3 butanediol in both the United States and Canada have contributed to a widespread interest in both the genera *Aerobacter* and *Aerobacillus*. The work reported in this series of papers has been chiefly on nutritional and bacteriophage problems that may affect the industrial potentialities of *Bacillus polymyxa* strains. The Division of Bacteriology and Dairy Research undertook these studies as their contribution in a co-operative investigation with the National Research Council. During the course of the work various factors affecting the growth of and fermentation with this organism were examined and, although a detailed study of these was not attempted, the results are of interest in comparison with those being published by Ledingham, Adams, and Stanier (1) on the fermentation of whole wheat mashes by *Bacillus polymyxa*.

Many factors influence the yield of any product of microbiological origin; the nature of the substrate, its composition, concentration, physical state, reaction; the type of organism or strain used, its history, activity, cultivation; and other factors such as temperature of incubation, time of fermentation, aeration and agitation, contaminations. All these conditions must be carefully adjusted so that maximum yields of the desired end-product may be obtained, at an economically practical level. The present paper deals with the influence of some of these factors on the fermentation of wheat by *B. polymyxa*.

[1] *Manuscript received February 24, 1944.*
Contribution No. 183 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Canada.
[2] *Agricultural Scientist.*

Experimental

In general, the procedures employed are based on those being published by Ledingham, Adams, and Stanier (1). A 15% mash (by weight) consisting of 17.8 gm. coarse whole wheat flour (No. 3 wheat), 1 gm. calcium carbonate and 100 ml. water was simultaneously cooked and sterilized for one hour at 15 lb. in a litre Erlenmeyer flask; after cooling it was inoculated with 10 ml. of a 48 hr. culture of the organism (in a starch–yeast–calcium-carbonate medium) shaken thoroughly and incubated for 48 hr. at 28° C. with vigorous agitation at 24 and 32 hr. The contents were filtered and the filtrate analysed for butanediol by extraction with *n*-butyl alcohol and oxidation with periodate. When required, analyses were made for ethyl alcohol by distillation and oxidation with dichromate. These procedures were made available through the courtesy of Dr. G. A. Adams of the National Research Council. In the text and all the tables the results are expressed as percentages by weight in the mash after fermentation.

Since a rapid fermentation was desired, it was decided to use a 48 hr. incubation period and adjust all conditions with a view of obtaining maximum yields in this period of time. It is believed that the high yields obtained in these experiments are primarily due to the use of 118.8 gm. of mash in litre flasks as compared with 300 ml. mashes in 500 ml. flasks used by the National Research Council. As will be shown later, the surface–volume ratio is of great importance in determining the diol–alcohol ratio; hence this rather minor difference in technique gives rise to somewhat different results. A 15% mash was considered the most useful from the standpoint of yield and ease of manipulation. The amount of carbonate added was found to be quite adequate and coarse flour equal to fine flour in yield capacity.

Influence of Amount and Age of Inoculum and Nature of Inoculating Medium

These three conditions were studied with results as presented in Table I. Neither the nature of the inoculating medium nor the age of the inoculum affected the fermentation. However a small amount of inoculum (0.5%) reduced the yield.

TABLE I

INFLUENCE OF AGE AND QUANTITY OF INOCULUM AND INOCULATING MEDIUM ON YIELD OF BUTANEDIOL BY STRAIN 47

Age inoculum (hr.)	Diol (%)	Amount inoculum (%)	Diol (%)	Nature of inoculating medium	Diol (%)
24	3.16	0.5	2.60	Yeast-starch-$CaCO_3$	3.09
48	3.21	2.5	3.04	Difco's M.R.V.-P.	3.13
96	3.17	5.0	3.04	5% whole wheat mash	3.08

Effect of Surface

As mentioned earlier experiments in the laboratories of the National Research Council with 300 ml. mash in 500 ml. Erlenmeyer flasks gave considerably lower yields of the diol than those indicated in Table I and suggested that surface might influence the reaction. It is well known that aeration is required in the *Aerobacter aerogenes* fermentation. In the absence of a suitable shaking apparatus, 4-litre Erlenmeyer flasks containing 500, 1000, 1500, and 2000 ml. mash were inoculated, incubated, and the resulting fermentation products analysed. It is clear from Table II that the yield of the diol, total products (diol + ethyl alcohol) and the diol–ethyl-alcohol ratio decrease as the amount of mash increases (and the surface is reduced).

TABLE II

INFLUENCE OF MASH SURFACE ON YIELD OF BUTANEDIOL AND ETHYL ALCOHOL BY STRAIN 47

Amount mash used (ml.)	Diol (%)	Ethyl alcohol (%)	Total products (%)	Diol / Alcohol
500	2.80	1.47	4.27	1.9
1000	2.65			
1500	2.49			
2000	2.04	1.33	3.37	1.5

Comparison of Activity of Different Strains

Eighty-two strains of *B. polymyxa* were made available by Dr. G. A. Ledingham of the National Research Council and were compared for their fermentative ability with regard to production of the diol. Marked differences were obtained varying from 0.2 to 3.5% diol with most of the cultures falling into the 2 to 3% group (Table III). Filtrability of the mashes varied strikingly; many were completely filtered in 5 to 10 min., others required several hours

TABLE III

YIELD OF BUTANEDIOL BY DIFFERENT STRAINS OF *B. polymyxa*

Number of strains	Diol (%)	Number of strains	Diol (%)
10	3.0 to 3.5	5	1.0 to 1.5
25	2.5 to 3.0	9	0.5 to 1.0
23	2.0 to 2.5	3	0.0 to 0.5
7	1.5 to 2.0		

and a number yielded 5 to 10 ml. of filtrate only after 5 to 6 hr. The hope that this property might be used as a strain characteristic was rather quickly dampened by the observation that several rapidly filtering strains gradually changed and produced mashes that filtered slowly. This is to be expected with such an unstable species as *B. polymyxa*. Further work on this aspect is being continued in the National Research Council Laboratories.

Use of Mixed Cultures

In view of the possibility of bacteriophage contamination and the resulting disruption of fermentation (a phase of this project that will be published in detail subsequently) experiments were designed using combinations of resistant and susceptible cultures, but keeping the total amount of inoculum constant. The results in Table IV indicate that mixtures of two strains gave high yields of the diol though no higher than the better yielding individual strains

TABLE IV

PRODUCTION OF BUTANEDIOL BY COMBINATIONS OF STRAINS OF *B. polymyxa*

Strain	Diol (%)	Strain combinations	Diol (%)
1	2.31	15 + 47	3.15
9	2.54	15 + 51	3.10
15	3.19	15 + 68	3.20
20	2.38	15 + 71	3.25
32	2.42	47 + 51	3.21
39	3.03	47 + 68	3.30
47	3.20	47 + 71	3.12
51*	2.85	51 + 68 + 71	2.80
68*	2.81	15 + 47 + 51 + 68 + 71	2.20
71*	2.98	All 10	2.27

* *Phage resistant.*

(No. 15 and 47). Combination of the three resistant strains also gave a good yield but when mixtures of 5 or 10 strains were used, the amount of diol produced dropped sharply.

Effect of Yeast Extract

According to the results obtained in the laboratories of the National Research Council (1) small amounts of yeast extract very slightly increased the amount of diol produced but with higher concentrations (1%) diol production was depressed. This work was repeated with many of the strains in the collection, 2 gm. of yeast extract being added to each flask before sterilization. The results with 12 of the best diol producers (Table V) indicate a consistent

TABLE V

EFFECT OF YEAST EXTRACT ON BUTANEDIOL PRODUCTION BY HIGH-YIELDING STRAINS OF *B. polymyxa*

Strain	Diol (%)		Strain	Diol (%)	
	Mash alone	Mash + yeast extract		Mash alone	Mash + yeast extract
10	2.92	3.44	45	2.92	3.15
15	3.21	3.53	47	3.40	3.67
35	3.03	3.27	51	2.79	3.01
36	3.11	3.49	68	2.75	3.27
39	2.83	3.40	71	2.88	3.31
41	3.17	3.43	82	3.06	3.29

increase in diol production. Some of the yields, especially those with strains 15 and 47 were unusually high. A second experiment was next set up in which different amounts of yeast extract, and the ashed, redissolved residue from 2 gm. of this substance were incorporated in the mashes. It appears from Table VI that 1 gm. of yeast extract gives maximum stimulation and

TABLE VI

EFFECT OF DIFFERENT AMOUNTS OF YEAST EXTRACT AND ASHED YEAST EXTRACT ON YIELD OF BUTANEDIOL AND ETHYL ALCOHOL BY STRAIN 15

Treatment	Diol (%)	Alcohol (%)	Diol / Alcohol
None	3.31	1.32	2.50
0.5 gm. yeast extract	3.56	1.28	2.78
1.0 gm. yeast extract	3.67	1.26	2.91
1.5 gm. yeast extract	3.62	1.20	3.02
2.0 gm. yeast extract	3.66	1.24	2.96
2.0 gm. yeast extract ashed and taken up in H_2SO_4	3.30	1.31	2.52

that ashing destroys the stimulatory fraction. The diol–alcohol ratio widened slightly with increasing amounts of yeast extract. Further work is planned on the effect of various components of yeast extract on this fermentation.

Influence of Different Grades of Wheat and Elevator Screenings on Yield of Butanediol*

Frozen wheat (No. 5 and 6) and other, poorer grades command from 13 to 17 cents per bushel less than the better wheats (No. 1 and 2). It is obvious then, that utilization of these lower grades would be more economical if their diol yield capacity were as high as that of the better quality wheats. A variety of good and "off grade" wheats and elevator screenings were tested, with two strains. There is no difference in the yield capacity of any of the wheats tested (Table VII), consequently the poorer grades should certainly

TABLE VII

FERMENTATION OF VARIOUS GRADES OF WHEAT AND ELEVATOR SCREENINGS BY STRAINS 1 AND 15

Sample	Diol (%)	
	Strain 1	Strain 15
No. 1 wheat	2.62	3.12
No. 2 wheat	2.61	3.24
No. 3 wheat	2.53	3.28
No. 5 wheat	2.48	3.22
No. 6 wheat	2.67	3.07
"Rejected heated"	2.64	3.22
"Feed"	2.57	3.01
"Smutty"	2.62	3.27
Uncleaned screenings	2.07	2.07
No. 1 feed screenings	1.19	1.89
Flax refuse screenings	1.12	1.38

* *The author is grateful to Mr. Johnston Edgar, Cereal Division, Central Experimental Farms Service, for suggesting this phase of the work.*

be given first consideration in the industrial development of this fermentation. Screenings, considerably lower in starch content, showed the expected drop in yield. However, the price of screenings is sufficiently low to warrant further investigation of this waste as a source of diol. A yield of 2% is possible; by selection of suitable strains and reinforcing the screenings with some of the lower grades of wheat higher yields may be obtained. The results of an attempt to fortify screenings with different quantities of a poor wheat are shown in Table VIII, from which it is obvious that yield of the diol may be increased by this treatment.

TABLE VIII

YIELD OF BUTANEDIOL BY STRAIN 1 AS INFLUENCED BY MIXTURES OF SCREENINGS AND "SMUTTY" WHEAT

Treatment	Diol (%)	Treatment	Diol (%)
1. "Smutty" wheat	2.58	8. Feed scr. + 20% 1	1.48
2. Uncleaned screenings	1.84	9. Feed scr. + 30% 1	1.68
3. Uncleaned scr. + 10% 1	1.91	10. Flax refuse screenings	1.14
4. Uncleaned scr. + 20% 1	2.04	11. Flax refuse scr. + 10% 1	1.14
5. Uncleaned scr. + 30% 1	2.21	12. Flax refuse scr. + 20% 1	1.27
6. Feed screenings	1.27	13. Flax refuse scr. + 30% 1	1.42
7. Feed scr. + 10% 1	1.33		

Discussion

A 3% butanediol fermentation from a 15% mash closely approaches the theoretical yield of this product (1). Under the experimental conditions described, such a yield has always been obtained with certain strains, alone or in combination with one or two others. Higher yields have also been realized, particularly in the presence of yeast extract. However, fermentation of large quantities of mash has consistently given low results. In view of the data in Table II it would seem that the amount of mash surface exposed is of prime importance. In large-scale operations the fermenting mixture can be agitated with stirrers or by compressed air although the excessive foaming attending violent agitation of wheat mash presents another practical problem.

Utilization of low-grade, low-quality wheats is to be recommended on the basis of the experimental results reported above. Screenings, a very inexpensive waste product, should also be considered as a source of the diol, and should be compared with wheat on a pilot-plant scale with particular emphasis on operation costs per unit of diol produced and from the standpoint of utilization of the spent mashes. Such considerations must receive careful attention if an economically practical fermentation is to be developed.

Reference

1. LEDINGHAM, G. A., ADAMS, G. A., and STANIER, R. Y. In preparation.

STUDIES WITH *BACILLUS POLYMYXA*

II. BACTERIOPHAGE OF *BACILLUS POLYMYXA* IN RELATION TO THE 2,3-BUTANEDIOL FERMENTATION[1]

By H. Katznelson[2]

Abstract

A procedure is described that resulted in the isolation of a number of phages against various strains of *Bacillus polymyxa*. Out of 82 strains tested, 63 were lysed completely and 6 partially. The phages produced plaques that were separated into eight morphological groups. No special relationship between susceptibility to phage and yield of butanediol was noted.

Temperature studies indicated that these phages were very sensitive, being inactivated by an exposure of 30 min. at 55° C. They were shown to be transmitted through the bacterial spore and under these conditions resisted a temperature of 80° C. for 60 min.

Eight out of 50 strains tested were lysogenic.

Phage was capable of almost completely stopping the fermentation of wheat mashes by susceptible strains. Certain resistant cultures gave good yields in the presence of active phage. Mixing resistant and susceptible strains resulted in lowered yields; however, a good fermentation was obtained in the presence of phage by combining three resistant strains. Even in the absence of phage, a mixture of five strains was not favourable, probably as a result of antibiotic effects among the individual strains. Secondary cultures differed markedly in nature of growth and spore-producing capacity from primary strains and were, on the whole, poorer diol producers.

It was suggested that under ordinary conditions in the factory, proper sterilization of equipment and materials, aseptic technique, and use of well-tested non-lysogenic strains are adequate precautions against this bacterial virus.

In contrast with its possible value as a therapeutic and prophylactic agent in medicine and public health (5, 10) phage is a decided liability in other fields in which bacteria play an important role: it is being recognized as one of the causes of failure of starter cultures in cheesemaking (7, 15); it has been reported as interfering with the butyl alcohol fermentation (12); and it is claimed to be responsible for decreased yields of legumes by destroying the root nodule bacteria (4, 8). The experiments reported here deal with the bacteriophage of *Bacillus polymyxa* in relation to the butanediol fermentation.

Experimental

Isolation and Activity of the Lytic Agent

A 10% whole wheat flour suspension containing 1% calcium carbonate was cooked for 15 min. and inoculated with moist compost soil, a possible source of phage, and with broth cultures of all strains of *B. polymyxa* being studied. After incubation at 28° C. for 48 hr. the mixture was filtered through paper, the filtrate adjusted to pH 6.5 and itself filtered through a sterile Seitz pad. In order to test for the presence of the lytic agent 3 ml. of this

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second filtrate was tested against a freshly inoculated 10 ml. culture of each strain in Methyl Red Voges-Proskauer (M.R.V.-P.) medium. These mixtures were incubated, examined after 24 and 48 hr. and filtered. Serial transfers were continued in this manner, the amount of filtrate tested being reduced with each passage, until transmissibility of the lytic agent (an essential criterion of phage activity) was definitely established. This required from 5 to 10 passages and is a necessary procedure since the original mash filtrate contains substances that inhibit bacterial growth but are diluted out by the serial passages.

At the same time the original mash filtrate suitably diluted was transferred to Petri plates containing a suspension of each bacterial strain, and 10 ml. of a 1% agar (M.R.V.-P.) medium was poured in. After incubation for 24 to 48 hr., plaques appeared, providing further proof of the presence of phage.

The data in Table I demonstrate the susceptibility of strains of *B. polymyxa* to freshly isolated phages and to a mixed phage preparation obtained from the Northern Regional Research Laboratory at Peoria, Ill. (through Dr. G. A. Ledingham of the National Research Council of Canada). Twenty active

TABLE I

LYSIS OF STRAINS OF *B. polymyxa* BY PHAGE ISOLATES

Strain No.	Source of phage		Strain No.	Source of phage	
	Soil	Old mixed phage		Soil	Old mixed phage
1	–	4	20	–	4
2	2	4	21	4	3
3	2	3	22	4	–
4	2	–	23	4	–
5	2	–	24	2	–
6	–	4	25	4	–
7	–	4	26	4	–
8	–	4	27	4	–
9	–	4	28	3	4
10	–	4	29	4	–
11	4	4	30	4	4
12	4	4	31	–	–
13	–	4	32	4	–
14	–	4	33	–	4
15	4	4	34	4	–
16	–	4	35	–	4
17	–	4	36	–	4
18	–	4	37	–	4
19	4	–	38	–	–

4 = *Complete clearing of culture.*
3 = *Almost complete.*
2 = *Distinct clearing.*
– = *No clearing.*

lytic agents were obtained in this investigation, 12 of which attacked strains resistant to the mixed phage preparation. Sixteen strains susceptible to the latter were resistant to the freshly isolated phages. Two cultures (31 and 38) were resistant to both.

Plaque studies with a number of the newly isolated lytic agents revealed distinct differences among them. The plaques differed in size and internal structure and were separated into eight groups, into which all subsequent phage isolates could be placed. Frequently one phage filtrate contained several different plaque-producing particles indicating that the filtrate was not pure. This demonstrates too that several phage variants can attack one bacterial strain, a fact that is in line with observations on animal and plant viruses in general.

Forty-four additional strains of *B. polymyxa*, freshly isolated, were obtained. The 82 cultures on hand were then tested against a phage preparation consisting of a mixture of all the phage isolates in the collection. Sixty-three strains were lysed completely, six partially. Of the susceptible strains 39 were sterilized completely, no secondary growth appearing even after two weeks; the remainder yielded secondary cultures that were isolated for further studies. Attempts were next made to isolate phages against the 13 resistant strains and four were obtained. It is quite conceivable that the remaining nine could be shown to have phages but this aspect of the work was not continued. No special relationship could be demonstrated between susceptibility to phage, and yield of butanediol from wheat (Table II).

TABLE II

RELATION BETWEEN SUSCEPTIBILITY OF *B. polymyxa* STRAINS TO PHAGE AND YIELD OF BUTANEDIOL

Range of butanediol (%)	Total number of strains	Susceptible	Resistant
3.0 +	8	8	0
2.5 – 3.0	31	27	4
2.0 – 2.5	24	18	6
1.5 – 2.0	7	5	2
1.0 – 1.5	5	5	0
0.5 – 1.0	3	3	0
0 – 0.5	4	3	1

Effect of Temperature on Phage

Phage contamination in factories is a danger that must be eliminated. Sterilization of equipment and of the fermentation substrate is, therefore, an essential procedure. However, the bacterial cultures themselves may be contaminated with phage that, under the conditions of the fermentation, might become active and harmful. Use of sporogenous strains and pasteurization of the inoculum might eliminate this danger although phage may be included in the spore and thus resist heat treatment as Cowles (2, 3) found with various other spore-forming bacteria.

Several of the secondary cultures isolated in the above studies were sporogenous and carried lytic agents to which they were resistant but that dissolved the original or primary cultures. These secondary strains retained their attached phages through many transfers and appeared to be truly lysogenic. One of these cultures (*S*55) was selected for the following temperature experiments.

(a) Heating of Filtrate—Culture *S*55 was inoculated into M.R.V.-P. medium and the turbid culture resulting was filtered after 24 hr. at 28° C.; this filtrate caused complete lysis of the primary strain (*P*55). The filtrate was next dispensed in 3 ml. amounts into sterile tubes that were immersed for various time intervals in a water-bath maintained at different temperatures. One ml. of the heated liquid was transferred into each of three tubes of M.R.V.-P. medium inoculated with the susceptible primary strain *P*55. The results presented in Table III show that the lytic principle is very sensitive to heat, being destroyed even at 55° C. for 30 min. This is an unusually low temperature of inactivation since the general impression is that phages are

TABLE III

INFLUENCE OF HEATING ON PHAGE

Exposure (min.)	Temperature of water-bath (°C.)				
	50	55	60	70	80
5	4	4	–	–	–
10	4	4	–	–	–
15	4	4	–	–	–
30	4	–	–	–	–
45	4	–	–	–	–
60	4	–	–	–	–

4 = *Complete clearing.*
– = *Turbid.*

somewhat more resistant to heat than bacterial cells. Sylvester (14) too found that phage against *Clostridium madisonii* was very labile, being inactivated by a five minute exposure at 65° C. Since much depends on the nature and reaction of the medium used it is difficult to compare the heat instability of phages for various organisms.

(b) Heating of Spore Suspension—A suspension of the secondary strain (*S*55) was prepared by washing old growth (consisting chiefly of spores) on agar slants with M.R.V.-P. medium; it was dispensed into sterile tubes and immersed in a water-bath as above. One loopful of the heated suspension was transferred to fluid medium and the culture incubated. If growth

occurred, the culture was filtered and the filtrate tested against the susceptible strain, *P*55. It may be seen in Table IV that a temperature

TABLE IV

INFLUENCE OF TEMPERATURE ON VIABILITY OF SECONDARY STRAIN OF *B. polymyxa* (*S*55) AND LYTIC ACTIVITY OF FILTRATE ON PRIMARY STRAIN (*P*55)

Exposure (min.)	80° C.		90° C.		100° C.	
	Viability of *S*55	Lysis of *P*55	Viability of *S*55	Lysis of *P*55	Viability of *S*55	Lysis of *P*55
5	+	4	0	–	0	–
10	+	4	0	–	0	–
15	+	4	0	–	0	–
20	+	4	0	–	0	–
30	+	4	0	–	0	–
40	+	4	0	–	0	–
60	+	4	0	–	0	–

+ = *Growth of secondary strain.*
0 = *No growth of secondary strain.*
4 = *Lysis of primary strain.*
– = *No lysis of primary strain.*

of 90° C. for five minutes inactivated both vegetative cells and spores, which, however, survived 80° C. (for 60 min.), a temperature that certainly must have destroyed any phage in the suspending fluid. However, the filtrates obtained from the cultures surviving this temperature were all active against the susceptible primary strain, from which it must be concluded that the phage may be transmitted through the spore (assuming that the vegetative cells had been killed by the heat) thus rendering pasteurization of inoculum for fermentation useless. In the light of these results it would be extremely difficult if not impossible to eliminate a contaminating bacteriophage from a culture.

Lysogenesis Among Strains of B. polymyxa

In the previous section it was shown that a contaminating phage cannot be eliminated from a culture by heat treatment without at the same time inactivating the culture carrying it. Since it is not considered a good policy to use such lysogenic cultures for fermentation, the strains employed should be carefully tested for the presence of a contaminating lytic agent. This is often very difficult. The procedure usually employed involves preparation of a broth filtrate of the suspected "carrier" strain and testing this filtrate on strains of known susceptibility. However, there is always the possibility that the test strains used are not susceptible to the particular lytic agent being carried by the suspected strain and thus the phage may escape detection. It then becomes a laborious process to obtain completely satisfactory evidence of the lysogenic nature of a culture. An attempt was made in these experiments to segregate the lysogenic strains in the collection. The filtrate from

each strain examined was tested on six of the most susceptible strains available. Of 50 cultures thus studied eight produced filtrates that were at least partially active against one or more of the test strains as may be seen in Table V, from which it also appears that Strain 82 is a particularly susceptible

TABLE V

LYSOGENESIS AMONG STRAINS OF *B. polymyxa*

Lysogenic strain	Susceptible test strain					
	2	15	28	40	65	82
5	–	–	–	–	–	2
21	–	–	–	–	–	2
34	–	?	?	–	–	2
36	–	?	–	?	–	?
38	–	2	–	–	–	2
49	–	2	–	2	–	2
53	–	–	–	–	–	2
66	–	–	1	–	–	–

2 = *Distinct clearing.*
1 = *Slight clearing.*
– = *No clearing.*

and useful test organism. Fortunately the strains that gave highest yields of butanediol did not produce phage against the six test strains. It is felt that such tests should be made before a strain is used in a fermentation; it should also be carefully observed during transfer for variation, or instability, characteristics frequently associated with the presence of phage.

Activity of Phage in Wheat Mashes

The seriousness of phage activity in a fermenting mixture may be readily noted from the following experiment. Litre Erlenmeyer flasks containing 17.8 gm. whole wheat flour, 1 gm. calcium carbonate, and 100 gm. water were sterilized for one hour at 15 lb. and inoculated with resistant or susceptible strains with and without active mixed phage filtrates. After 48 hr. at 28° C. the contents were filtered and the filtrate analysed for butanediol and ethyl alcohol (11). The results are given in Table VI. The marked reduction

TABLE VI

INFLUENCE OF PHAGE ON YIELD OF BUTANEDIOL AND ETHYL ALCOHOL BY STRAINS OF *B. polymyxa*

Strain	Butanediol (%)		Ethyl alcohol (%)	
	No phage	Phage added	No phage	Phage added
1	2.35	0.19	1.04	0.13
15	3.12	0.63	1.24	0.13
47	3.13	0.18		
51	2.83	2.62		
68	2.85	2.86		
71	2.98	1.76		

in yield of fermentation products as a result of phage action on susceptible Strains 1, 15, and 47 is quite apparent. Strains 51 and 68 are completely resistant whereas Strain 71 is somewhat susceptible. The inference from these results is that resistant strains should be used in the fermentation. Although this is a fairly good general rule, it must be pointed out that such strains can lose their resistance after a number of transfers. Furthermore, strains resistant to one phage may still be susceptible to others that may be introduced accidentally into the factory (14). Again, an examination of the cultures in the collection showed that the most active and therefore the most useful fermenters (Nos. 15 and 47) happened to be susceptible although as pointed out previously this is not a general rule by any means. Nevertheless, resistant strains yielding 3% diol or more would be very desirable.

It might be assumed that a mixed culture composed of high yielding susceptible and resistant strains would assure a good fermentation. Accordingly an experiment was planned with the above resistant and susceptible cultures in combinations as illustrated in Table VII. The resulting data are interesting from a theoretical as well as a practical point of view. The two susceptible strains in combination suffer inhibition by phage equivalent to that of

TABLE VII

EFFECT OF PHAGE ON YIELD OF BUTANEDIOL BY SINGLE AND MIXED CULTURES OF SUSCEPTIBLE AND RESISTANT STRAINS OF *B. polymyxa*

Strains	Butanediol (%)	
	No phage	Phage added
15	3.29	1.07
47	3.37	0.20
51	2.80	2.50
68	2.81	2.82
71	2.88	1.30
15 + 47	3.15	0.27
15 + 51	3.10	1.61
15 + 68	3.25	1.34
15 + 71	3.25	0.73
47 + 51	3.21	1.12
47 + 68	3.33	1.73
47 + 71	3.12	1.14
51 + 68 + 71	2.80	2.79
15 + 47 + 51 + 68 + 71	2.20	2.00

the more susceptible strain, No. 47. Similarly, combinations of susceptible and resistant cultures lead to a greater suppression of fermentation by phage than might be expected, considering the resistance and yield of cultures 51 and 68. This aspect of bacteriophagy has been a controversial issue for many years. Bronfenbrenner (1) suggests that resistant bacteria remain unaffected even though lysis of susceptible bacteria takes place. On the other hand, Wollman and Wollman (16) reported the ability of phage to lyse resistant strains of staphylococci in the presence of living susceptible

strains. Furthermore, Evans (6) found a resistant strain of streptococcus to be sensitive to the lytic principle in the presence of a growing culture of a sensitive strain. These findings are supported by Meanwell (13) working with starters for cheesemaking (lactic acid streptococci). The present experiments with *B. polymyxa* give further support to these observations (Fig. 1).

Combination of the three resistant strains provided a satisfactory inoculum in the presence of the lytic agent though the yield is lower than that produced by the single susceptible strains. It is interesting to note that a mixture of the two susceptible and three resistant strains, though not appreciably affected by phage is nevertheless a poor inoculum from the standpoint of yield of butanediol owing probably to some antagonism among the strains.

Fermentation by Secondary Cultures

It was pointed out earlier that certain strains in the collection produced secondary growth in tubes originally cleared by phage. These secondary cultures were found to differ appreciably from the primary cultures, usually producing a flat, dry, dull wrinkled growth on slants in place of the glistening, mucoid appearance of most of the primary cultures. They also differed from the latter in spore-producing capacity as the data in Table VIII show.

TABLE VIII

SPORULATION AND YIELD OF BUTANEDIOL BY PRIMARY AND SECONDARY CULTURES OF *B. polymyxa*

Strain	Sporulation		Butanediol (%)	
	Primary	Secondary	Primary	Secondary
18	−	1+	2.84	2.60
24	−	−	1.31	1.70
26	3+	−	2.11	2.23
27	2+	−	2.24	2.33
32	2+	1+	1.86	1.11
34	1+	3+	1.11	0.98
38	4+	3+	2.70	1.32
44	−	−	2.08	1.61
53	−	4+	1.12	0.51
54	−	−	1.37	0.20
56	−	−	2.54	0.68
57	−	−	2.65	2.52
58	−	2+	2.10	1.50
62	−	−	1.57	1.45
69	−	−	0.83	0.91
77	1+	4+	1.39	2.09
79	1+	1+	0.54	0.10

1+, 2+, 3+, 4+ = *Degree of sporulation.*
− = *No sporulation*

Because of these differences it was considered of interest to compare their fermentation characteristics with those of the original strains. As indicated in Table VIII none of the secondary strains is particularly outstanding in this connection; in fact they are, on the whole, inferior to the primary strains.

PLATE I

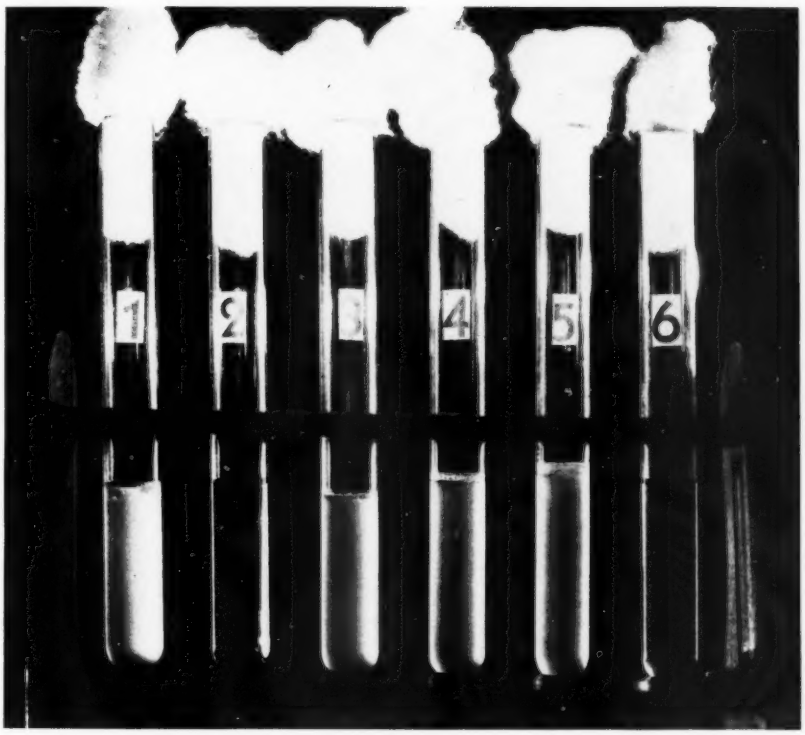

FIG. 1. *Lysis of resistant strain of Bacillus polymyxa in presence of a susceptible strain. 1. Susceptible strain No. 1. 2. Susceptible strain No. 1 + phage—culture lysed. 3. Resistant strain No. 68. 4. Resistant strain No. 68 + phage. 5. Susceptible + resistant strain. 6. Susceptible + resistant strain + phage—culture lysed.*

Discussion

Bacteriophage is often very difficult to control once it gains a foothold in a factory. Equipment, fermentation substrate, personnel, and the cultures themselves may carry phage thereby making its eradication almost impossible. Sterilization of equipment and materials is an essential control measure as McCoy (12) has pointed out. She also suggested "use of mixed cultures, increased percentage of inoculum or use of phage-resistant strains purposely prepared". Mixed cultures may be used but the mixture must be carefully prepared to consist of high-yielding strains that do not exert antibiotic effects against one another, thereby causing a reduction in yield (Table VII) (9). Increased percentage of inoculum may also be useful, yet in the mash experiments reported above and in others not mentioned an inoculum of 5 to 10% was used without affecting phage activity. Larger amounts of inoculum would hardly be economical. Resistant strains have been successfully used (Tables VI and VII), but it is quite possible that new phages to which the resistant cultures in use are susceptible may be introduced into the factory (14). An inoculum consisting of a mixture of high yielding resistant, non-lysogenic strains is probably the best solution of the problem should it become particularly serious. However, under ordinary conditions, proper sterilization of the equipment and materials, aseptic technique, and use of well-tested, non-lysogenic strains are considered to be adequate precautions.

APPENDIX

LIST OF CULTURES ON HAND

Serial No.	Isolate	Serial No.	Isolate	Serial No.	Isolate	Serial No.	Isolate
1	510	22	*C*15(1)	43	*C*2(1)	64	*C*37
2	8*A*1	23	*C*15(2)	44	*C*2(2)	65	*C*39
3	*CS*1	24	*C*17(1)	45	*C*3(1)	66	*C*40(1)
4	206*U.A*1.	25	*C*17(2)	46	*C*3(2)	67	*C*40(2)
5	211*U.A*1.	26	*C*18(1)	47	*C*3(3)	68	*C*42(3)
6	233*a*(1)*U.A*1.	27	*C*18(2)	48	*C*7	69	*C*45(1)
7	233*a*(2)*U.A*1.	28	*C*19	49	*C*8(1)	70	*C*45(2)
8	233*a*(3)*U.A*1.	29	*C*20	50	*C*8(2)	71	*C*46(3)
9	233*b*(1)*U.A*1.	30	*C*23	51	*C*8(3)	72	*C*47(1)
10	233*b*(2)*U.A*1.	31	*C*24	52	*C*9	73	*C*47(2)
11	*C*2*a*	32	*C*25	53	*C*10	74	*C*48(1)
12	*C*4(1)	33	*C*26	54	*C*12	75	*C*48(2)
13	*C*4(2)	34	*C*31	55	*C*13	76	*C*49
14	*C*4(3)	35	*C*38(1)	56	*C*14(1)	77	*C*51
15	*C*5(1)	36	*C*38(2)	57	*C*14(2)	78	*C*53
16	*C*5(2)	37	*C*42(1)	58	*C*16(1)	79	*C*55
17	*C*6(1)	38	*C*42(2)	59	*C*16(2)	80	*C*56(1)
18	*C*6(2)	39	510*R*	60	*C*21	81	*C*56(2)
19	*C*7	40	510(1)	61	*C*22(1)	82	233(1)
20	*C*9	41	510(2)	62	*C*22(2)		
21	*C*12	42	*C*(1)	63	*C*29		

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A CULTURAL AND CYTOLOGICAL INVESTIGATION OF A TWO-SPORED BASIDIOMYCETE, *ALEURODISCUS CANADENSIS* n. sp.[1]

By A. J. Skolko[2]

Abstract

A new species of *Aleurodiscus* is described and designated *Aleurodiscus canadensis*. The basidia of this fungus are regularly two-spored, but occasionally basidia bearing three spores or rarely one spore are encountered. From the cultural and cytological evidence presented the normal life cycle may be described as follows. The cells of the subhymenium are dikaryotic with clamp connections. The young basidia are at first binucleate. Following karyogamy the fusion nucleus undergoes reduction division producing the usual tetrad of nuclei. Each spore of the two-spored basidium receives two of the four daughter nuclei. The two nuclei in these spores undergo further division while the spore is still attached or shortly after discharge, so that the mature spore contains four nuclei. Such a spore is capable of producing a mycelium bearing clamp connections. Although the majority of the spores produce mycelia with clamp connections, a spore may occasionally develop a mycelium that does not bear clamps. When such exceptional mycelia are mated in compatible combinations, dikaryotic mycelia with clamp connections are obtained. From the mating of a number of these exceptional haplonts the heterothallic and bipolar relationship is made evident. Although complete cytological information on the spores that give rise to these exceptional haploid mycelia is lacking, it is probable that these spores are originally uninucleate and that this results from the distribution of the four nuclei in a three-spored basidium in such a way that one spore receives two nuclei and the other two spores one nucleus each. The two nuceli that migrate into the normal spore, therefore, probably bear allelomorphic interfertility factors. If this species can be considered homothallic, the homothallism is a different type from that found in species that complete their life cycle from the development of a single uninucleate spore. The necessity for such a distinction is stressed.

In the Hymenomycetes the study of the cytology of two-spored forms by several early investigators was more or less incidental to investigations of four-spored forms and, as a result, the observations were frequently fragmentary and contradictory. More recently, with the realization of the theoretical possibilities of variation in nuclear cycle accompanying reduction of spore number, more intensive cytological research has been devoted to these forms. Following the discovery of heterothallism in the Hymenomycetes by Bensaude (6) and Kniep (32), and through subsequent cultural studies by other investigators, it became possible to correlate the cytological and genetical phenomena in normal, four-spored, heterothallic species. With but few exceptions cultural studies have not been undertaken in order to correlate the inheritance of genetic factors, especially factors of interfertility, with observed cytological phenomena in two-spored forms. This general lack of cultural data is, in part, due to the fact that many of the species do

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not lend themselves to cultural manipulation. When an apparently undescribed species of *Aleurodiscus* having normally two-spored basidia and with regular clamp connections on the mycelium came into the hands of the writer, it seemed desirable material for a detailed cultural study.

Bauch (4) has prepared a list of Basidiomycetes, exclusive of the Protobasidiomycetes, in which odd-spored basidia have been reported. To this list Buhr (10) has made a number of additions. These include a number of records taken from descriptions in taxomomic works where many species are listed as having two to four sterigmata. Had Buhr consulted the work of Bourdot and Galzin (8) and other similar descriptive works, the lists might have been greatly augmented. Species in which two-spored basidia are the rule are quite common and are known in widely separated genera scattered throughout the Hymenomycetes. They are the rule in the family Dacryomycetaceae. Often the two-spored character has been used as the primary character to separate species. In the Agaricaceae many bispored forms of species that regularly develop four spores have been observed. Some taxonomists have recognized these bispored forms as species. Others have questioned this procedure.

Even though variation in the number of spores from the normal four is not infrequent in many Basidiomycetes, it should be recognized that in the great majority of such cases these variations may be of little biological significance and that many of the cases of variation recorded in descriptive works, particularly of those listed as having two to four sterigmata, may often be the result of faulty observation. It is when a number other than four occurs with considerable regularity or is the "normal" condition in a genus, species, or race that any great significance need be attached to the phenomenon. Where the number is greater than the usual four spores, equational divisions following the normal reductional divisions and preceding nuclear migration to the spores, will account for the required increase in the number of nuclei. When, however, the number of spores developed on a basidium is less than four, several possibilities must be considered with respect to the sequence of nuclear events. While it is true that one- and three-spored basidia do occur, except in a few one-spored species, these do not occur with any regularity and are usually observed in species that are regularly two- or four-spored.

To review the cytological data that have so far been recorded in forms with reduced spore numbers, it is convenient to group them around four types of nuclear sequence that appear to be distinct though perhaps not all of equal

significance. In this review only the homobasidial forms, inclusive of the Dacryomycetaceae, are considered. Jackson (28) has recently summarized most of the variations known to occur in the groups having septate basidia (Heterobasidiomycetes) and these need not be included here.

TYPE I: According to this type the young basidium contains the dikaryon, nuclear fusion occurs, the fusion nucleus undergoes meiosis through two divisions, the resulting four daughter nuclei divide again—thus the mature basidium contains eight nuclei, one nucleus migrates into each of the two spores, the residual nuclei in the basidium degenerate.

Examples: *Clavaria rugosa* Bull., Maire (37), Bauch (5); *Clavaria grisea* Pers., Maire (37); *Clavaria cristata* Fr., Bauch (5); *Clavaria cinerea* Fr., Juel (30), Bauch (5); *Craterellus cornucopioides* L., Rosenvinge (42), Juel (30), Buhr (10).

TYPE II: In this type the young basidium is binucleate, the two nuclei fuse, the fusion nucleus undergoes meiosis through two divisions that result in the usual four daughter nuclei, then (*a*) a third division, which may be irregular, results in five to eight nuclei in the mature basidium; two of these migrate to each spore and the residual nuclei degenerate in the basidium, or (*b*) of the four basidial nuclei, two migrate directly to each spore and there undergo the third division—the mature spore thus contains four nuclei and no residual nuclei are left in the basidium.

Examples: *Mycena metata* Fr., *M. clavicularis* Fr., *M. graveolens* Kauff. & Smith, *M. immaculata* Pk., *M. margaritispora* Lange, *M. mirata* Pk., and *M. viscosa* Maire, Smith (51).

TYPE III: The young basidium is binucleate, the two nuclei fuse, reduction division results in four daughter nuclei, either (*a*) one nucleus migrates to each spore and the residual two nuclei degenerate or (*b*) two nuclei migrate to each spore.

Examples of Type III*a* are *Dacryomyces deliquescens* Bull., Juel (29), Maire (37); *Dacryomyces chrysocomus* Bull., Istvanffi (27); *Dacryomyces abietinus* Pers., Buhr (10); *Guepinia Spathularia*, Bodman (7); unspecified *Dacryomyces* spp., Wager (55), Gilbert (26); *Pholiota erebia* Fr. and *Nolanea cetrata* Fr., Buhr (10); possibly also *Amanita bisporigera* Atkinson, Lewis (36). Other species have been reported to follow both III*a* and III*b* as for example *Psalliota campestris* Quel., Buhr (10), Sarazin (44); *Mycena debilis* Fr., *M. hiemalis* (Osbeck) Fr., and *Galera triscopa* Fr., Buhr (10).

Maire (37) claimed that, in *Psalliota campestris* Quel., each spore receives two nuclei but often the second division does not take place in the basidium and each spore then receives only one nucleus, which undergoes the second division in the spore. Sass (45) corroborated the results obtained by Maire but mentions that there is slight evidence that in some basidia only one division occurs. The migration of two nuclei to each spore is also reported by Colson (15) but no mention is made of the possibility that only one nucleus may pass into each spore. Colson's observations are supported by those of Kligman (31).

Sass (46, 47) noted that in the two-spored forms of *Coprinus ephemerus* Fr., *Galera tenera* Fr., and *Naucoria semiorbicularis* Fr., of the four daughter nuclei resulting from the reductional divisions, normally two nuclei pass to each of the two spores. Occasionally, he claims, a spore may receive only one nucleus. His cultural data substantiate this claim, but the manner in which this condition might arise was not determined.

In *Hydnangium carneum* Wallr., Ruhland (43) reported that either two nuclei may migrate into each spore or that three nuclei may migrate into one spore and one into the other spore.

The condition reported by Maire (37) in *Dacryomyces deliquescens* Bull. and *Calocera cornea* Pers., in which each spore receives one of the four daughter nuclei resulting from reduction of the fusion nucleus and two spore generations are produced, has not been verified. Similarly, the occurrence of a single division of the fusion nucleus in the basidium of *Dacryomyces deliquescens* Bull., *Calocera viscosa* Pers., Dangeard (16), *Calocera cornea* Pers., and *Psalliota campestris* Quel., Maire (37), is doubtfully received.

TYPE IV: The young basidium is uninucleate, only one division occurs, each spore receives one of the daughter nuclei.

This type is referred to as "haploid parthenogenesis" by Bauch (4). To this type belong *Hygrocybe conica* Scop., Maire (37), Fries (25), *Camarophyllus virgineus* Wulf., Bauch (4), *Mycena galericulata* (Scop.) Fr., Kühner (34), Buhr (10), *Mycena megaspora* Kauff., *M. alcalina* Fr., *M. capillaris* Fr., *M. cholea* Smith, *M. citrinomarginata* Gillet, *M. dissiliens* Fr., *M. lasiosperma* Bres., *M. leptocephala* Fr., *M. polygramma* Fr. var. *albida* Kauff., *M. roseipallens* Murr., *M. rubromarginata* var. *Laricis* Smith, and *M. vitilis* Fr., Smith (51).

These two-spored species, forms, or variants may possibly be fruiting haplonts of normally heterothallic species that have been isolated through loss of fertility as a result of mutation. Smith (51) attempted by cultural

means to determine whether they are haplonts of heterothallic species but failed to obtain evidence of successful mating.

In spite of the fact that one- and three-spored basidia do occur in some of the species mentioned above that have been studied cytologically, information on the nuclear behaviour in such odd-spored basidia is notably lacking. This is not entirely surprising when it is considered that odd-spored basidia in these species are rather rare and the possibility of observing their nuclear condition in a cytological preparation is unlikely. In a few cases such observations have been possible. In *Mycena polygramma* Fr. var. *albida* Kauff. Smith (51) described the nuclear behaviour in three-spored basidia. The two-spored form of this species is "haploid parthenogenetic". If three sterigmata are formed, either two nuclei migrate each into a spore leaving the third spore without a nucleus or, as is usually the case, one of the nuclei divides at the base of a sterigma. Thus three nuclei are contained in the basidium and one nucleus passes to each of the three spores. Maire (37) suggested this latter possibility for *Hygrocybe ceracea* where the basidium was found to contain three nuclei one of which passed to each of the three spores; the origin of the three nuclei, however, was not ascertained. He did state that these three basidial nuclei probably resulted from the division of one of the two daughter nuclei. The only other mention of nuclear behaviour in three-spored basidia is the conjecture of Buhr (10) concerning the condition in *Pholiota erebia* Fr. He suggested that spore production in three-spored basidia of this fungus probably followed the usual scheme (namely, two divisions of the fusion nucleus produced four daughter nuclei and one nucleus passed to each spore), except that the basidium must contain one degenerating nucleus after spore discharge.

The only information of nuclear development accompanying spore production in one-spored basidia is that presented by Ruhland (43) for *Hydnangium carneum* Wallr. He found that the four nuclei resulting from two divisions of the fusion nucleus all pass into the single spore.

In connection with his cytological investigations in several species of *Mycena*, Smith (51) conducted a cultural study for *Mycena megaspora*. His cytological observations indicated that this species is haploid parthenogenetic. He attempted to obtain diploidisation by mating monosporous mycelia but without success. Fifty single spore isolations were made from fruit bodies collected in different localities and the mycelia from them were mated but no clamp connections were produced. Since, however, the dikaryophase may be established even in the absence of clamp connections, he examined the mycelia derived from the mating cytologically but in no matings were dikarya observed.

Colson (15) realized the need of genetical data in her cytological investigation of *Psalliota campestris*. She stated that "the occurrence of initially uninucleate and initially binucleate spores, within one species, immediately raises questions as to the presence of heterothallism and homothallism in the four-spored and two-spored varieties respectively." Kligman (31) has made a cytological examination of monosporous mycelia and of tissue of sporophores of monosporous origin in the two-spored or cultivated form of *Psalliota campestris*. He found no evidence of conjugate nuclei but since monosporous mycelia "normally" give rise to fruiting bodies in this form he holds that it must be considered homothallic.

Sass (46, 47) has provided some information on homothallism and heterothallism in two-spored forms from his study of the four- and two-spored forms of *Coprinus ephemerus* Fr., *Naucoria semiorbicularis* Fr. and *Galera tenera* Fr. The four-spored form of *Coprinus ephemerus*, which follows the normal scheme of nuclear behaviour—binucleate young basidium, nuclear fusion of the two basidial nuclei, reduction division and migration of one of the four daughter nuclei to each of the four spores—he found to be heterothallic and bipolar. That is, haploid mycelia derived from monosporous isolations will develop the diploid phase (or dikaryophase) only when mated in compatible combinations; a single pair of allelomorphic factors governing interfertility segregated the haploid mycelia into two groups. The two-spored forms of all three species are apparently homothallic, i.e. monosporous isolations when grown on agar ordinarily produce a clamped (dikaryotic) mycelium and fertile fruit bodies. In *Coprinus ephemerus* forma *bisporus* about 10% of the viable monosporous mycelia remained in the primary condition (without clamp connections) and in *Galera tenera* f. *bispora* about 4.5%. Pairing these exceptional primary (haploid) mycelia in all possible combinations gave compatible reactions as indicated by the development of secondary mycelia with clamp connections in a few of the matings. This phenomenon Sass called "aberrant heterothallism" but further stated that "segregation into sex classes is not evident" and that "it is probable that more than one pair of factors are involved." It must be noted that in these mating experiments Sass used an admittedly small number of primary monosporous isolates, in one experiment 9, in another 13, and in the third only 6. He was able, however, to establish the existence of heterothallism within what appeared to be normally two-spored homothallic species. This subject will be discussed after the results of the present study have been presented. The nuclear basis for this condition, as has been indicated under Type III, has

been described by Sass. Normally each spore receives two nuclei, which he assumes carry "allelomorphic sex factors". There is evidence that occasionally a spore receives only one nucleus and hence produces a haploid mycelium.

A comparable situation to that reported by Sass in *Coprinus ephemerus*, etc., exists in certain Ascomycetes with reduced spore numbers. The evidence in these cases is more conclusive. In the genus *Neurospora*, Shear and Dodge (50) found the eight-spored species, *Neurospora sitophila* and *N. crassa*, to be heterothallic and the four-spored species, *N. tetrasperma*, to be homothallic. The nuclear phenomena underlying heterothallism and homothallism in these species have been reported by Dodge (18, 19). In the heterothallic species each spore contains a single haploid nucleus whereas in the homothallic species, *N. tetrasperma*, normally two non-sister nuclei co-operate in delimiting each ascospore. Occasionally, two adjacent nuclei fail to co-operate in cutting out a spore and each delimits a dwarf spore. Monosporous mycelia from these dwarf uninucleate spores produce only conidia but, when properly mated, will develop ascocarps. These monosporous mycelia fall into two groups on the basis of their mating reaction (50). Dodge claims, therefore, that ". . . . . the existence of pseudo-heterothallism [as he refers to this phenomenon in *N. tetrasperma*] is based on the accidental inclusion of only one of the two sexually different nuclei which are normally contained in each spore at its origin." Similar conditions in the Ascomycetes *Pleurage anserina* (Ces.) Kuntze, Dowding (23) and Ames (1, 2), *Gelasinospora tetrasperma*, Dowding (24), "*Sordaria fimicola*,"[1] Page (38), and *Podospora minuta*, Page (39) have been reported.

Materials and Methods

In late June of 1938 fresh material of a collection of an *Aleurodiscus* on branches of *Picea canadensis*, made by Professor H. S. Jackson at Burnet, Que., was examined by the writer. This collection showed that the basidia commonly bore two spores, occasional basidia bearing one or three spores. The hyphae of the subiculum showed abundant clamp connections. The basidia and spores were of large size and the species seemed to offer suitable material for a detailed cultural and cytological study. Such a study was undertaken with the hope that further information might be obtained with reference to the sequence of nuclear events in two- and three-spored basidia. The results of this study provide the basis of the present contribution.

Careful comparison with other known species of *Aleurodiscus* has led to the conclusion that this form on *Picea*, which has since proved to be quite common in certain sections of Ontario and Quebec, represents an undescribed species, a description of which is here presented.

[1] *Probably a geographical race of Gelasinospora tetrasperma (24, footnote p. 295).*

Aleurodiscus canadensis n. sp.

Frutificatio resupinato, effuso; hymenio levi, niveo in humido; hyphis nodoso-septatis; paraphysibus moniliformibus et penicillatis; basidiis clavatus, 48 − 56 × 11 − 13 μ, 2-sporis, interdum 1- vel 3-sporis; sporis hyalinis, oblongo-ellipsoideis, levibus, 15 − 18 × 10 − 13 μ, basi distincte apiculatis.

Hab. ad cortices in latera inferiore ramorum emortuorum *Piceae canadensis* vivae.

The fruiting bodies (Plate I, Fig. 1) are entirely resupinate, effused, and closely adnate to the substrate. The surface of the fructification is smooth but often cracks in drying. In colour it is pure white when fresh but may become light buff when dry. The margin when mature is determinate and the fructifications vary from small isolated patches to extensive areas, sometimes covering the entire under surface of the twig. In structure it is 60 − 150 μ thick and is composed of densely interwoven hyaline hyphae, 2.5 μ in diameter and with abundant clamp connections at the septa. There is no appreciable amount of incrustation. The hymenium (Plate I, Figs. 2 to 5) is composed of basidia and two types of paraphyses. The moniliform paraphyses or pseudophyses, as they are referred to by Pilat (40), have one to several constrictions and densely granular contents. Paraphyses of the second type (called acanthophyses by Pilat) are penicillate or "bottle-brush"-shaped as described by Burt (14). They vary from those that are narrow and hyphal-like with numerous hyaline spine-like projections to those of broad basidial form with only a few reduced spines at their apex. The basidia are clavate, 48 − 56 × 11 − 13 μ with usually two, occasionally three, and rarely one, stout, curved sterigmata. Some collections show the three-spored character abundantly while in others it is rare. The spores are hyaline, smooth, thin-walled, oblong-ellipsoid, 15 − 18 × 10 − 13 μ and are conspicuously apiculate.

The fruiting bodies are found on the bark of dead twigs and branches, confined to the underside, in the lower part of living white spruce. They are to be found from late June to the middle of September.

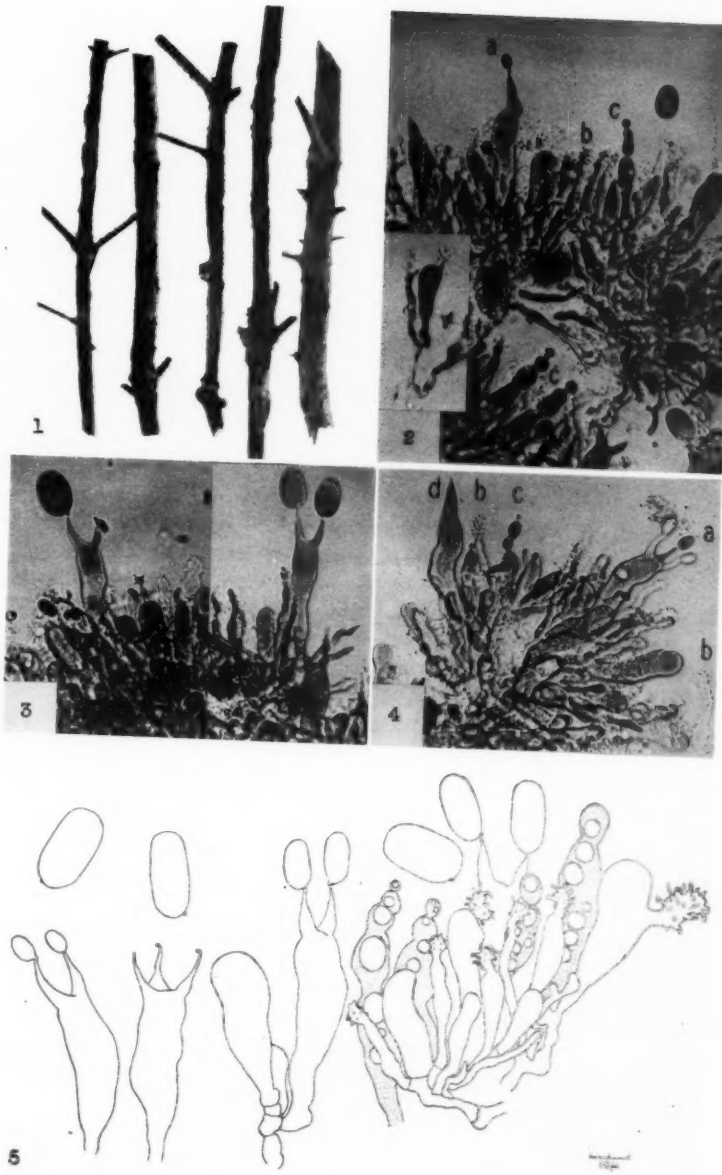
TYPE: On *Picea canadensis* (Mill.) BSP, Burnet, Que., July 1942, A. J. Skolko (*TRT* 18147).[1]

[1] *TRT—Herbarium of the University of Toronto; LOO—Herbarium of L. O. Overholts, State College, Pennsylvania; DAOM—Mycological herbarium of the Department of Agriculture, Division of Botany and Plant Pathology, Central Experimental Farm, Ottawa.*

EXPLANATION OF PLATE I

FIG. 1. *Aleurodiscus canadensis. Fruiting bodies on the underside of twigs of white spruce.* FIG. 2. *Microphotograph of crushed hymenium showing (a) young one-spored basidium, (b) penicillate paraphyses, (c) moniliform paraphyses.* FIG. 3. *Microphotograph of crushed hymenium showing mature two-spored basidia. Note the unequal development of the spores on the basidium on the left. One spore has failed to develop and probably would remain abortive.* FIG. 4. *Microphotograph of crushed hymenium showing (a) young three-spored basidium, (b) penicillate paraphyses, (c) moniliform paraphyses with granular contents, and (d) probably a young one-spored basidium.* FIG. 5. *Camera lucida drawing of crushed preparation of the hymenium showing two- and three-spored basidia and paraphyses.*

PLATE I



SPECIMENS EXAMINED: On *Picea canadensis* (Mill.) BSP or on *Picea* sp. QUEBEC: Burnet, June 1938, H. S. Jackson (*TRT* 18663); Burnet, July 1938, F. L. Drayton (*TRT* 18661) and Aug. 1938, (*TRT* 18662); south of Laurentide National Park, Aug. 1938, H. S. Jackson (*TRT* 18660); Duchesnay, Aug. 1938, H. S. Jackson (*TRT* 18659); Duchesnay, Aug. 1938 (on *Abies?*), L. O. Overholts (*LOO* 21417); St. Martin, Ile Jesus, near Montreal, Aug. 1941, H. S. Jackson (*TRT* 18657); Tadoussac, Aug. 1943, A. J. Skolko (*DAOM* 11270). ONTARIO: Algonquin Park, Sept. 1938, H. S. Jackson (*TRT* 13454) and Sept. 1939, (*TRT* 14872); Chalk River, Sept. 1941, H. S. Jackson (*TRT* 17500).

On *Thuja occidentalis* L., Burnet, Que., June 1938, H. S. Jackson (*TRT* 18658).

Smith (51) made a comparative study of spore size in a number of typical (four-spored) species of *Mycena* and with their variant (two- and three-spored) forms. In some species he found that there was little difference in size or shape between the typical and variant forms. In others there was some increase in size in the variant form and this increase might or might not be accompanied by change of spore shape. In *Aleurodiscus canadensis*, from the measurement of 60 detached spores, it was found that there is considerable variation in spore size, ranging from $10 - 19 \times 7 - 16\ \mu$ with an average of $16 \times 11\ \mu$. Examination of attached spores on one- and three-spored basidia did not give sufficient evidence for correlating size of spores with reduction or increase of spore number. So far no related four-spored form of this species has been found.

The material used in both the cultural and cytological investigations was obtained from collections[1] made at Burnet, Que., where the fungus fruits abundantly each summer. Active sporulation of the fructifications was induced by placing the twigs in a moist chamber for 8 to 10 hr. Single spore isolations were obtained from a scattered spore deposit on filtered malt agar. The spores were allowed to germinate before being isolated. There is apparently little difference in the mycelial development on potato-dextrose or malt agar. For the cytological study the material from the moist chambers was fixed in several solutions of which La Cour's 2BE, chrom-acetic acid, and formalin acetic alcohol gave good results. Wakayama (56) found that, in a number of species of the Agaricaceae, nuclear division was more active at night. In view of Wakayama's results, fixation was carried out during the day and also at 3 a.m. No periodicity of division was found in the present material. Embedding was carried out in the usual way. Sections were cut from 5 to 15 μ in thickness. For detailed nuclear structure the thinner sections are to be preferred, but they often show only a portion of the basidium. The sections were stained with Flemming's triple stain, Haidenhain's haematoxylin, or safranin, crystal violet, and a fast-green counterstain, or with

[1] *The first of these was kindly made by Dr. F. L. Drayton during the late summer of 1938, the second by the writer in July, 1940.*

safranin and fast-green alone. In the course of the morphological studies of the hymenium, mounts of living material, crushed in a weak aqueous solution of phloxine (about 2%) and 10% potassium hydroxide solution, were used. It was found that in such mounts the phloxine acted as a weak nuclear stain and, although phloxine-stained material was not used for critical observation (since nuclear definition was vague at best) examination of such material corroborated other nuclear counts.

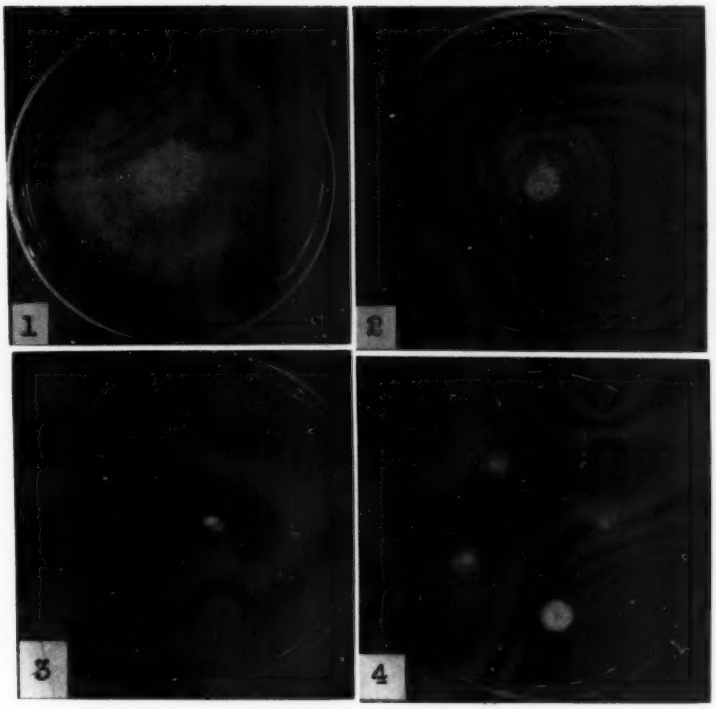
Cultural Data

Most of the spores germinate readily on malt agar, usually within a few hours at room temperature. A germ tube is put out from one or both ends of the spore and may soon branch or continue to grow for some time without branching. Clamp connections appear even before the colony becomes macroscopically visible, usually within 24 hr. The mycelium grows slowly forming a white colony with the hyphae for the most part submerged in the medium or appressed to the surface of the agar. Relatively little aerial growth takes place.

Some spores failed to germinate, at least up to the time when the single spore isolations were made. Of the first 240 single spores isolated, 222 produced clamp-bearing mycelia or failed to develop. Actually only a small percentage did not develop but it should be noted that they had already germinated before isolation. No record of the number that failed to develop was made. Since the majority of spores produce mycelia with clamp connections, the species is apparently homothallic. The remaining 18 monosporous mycelia did not produce clamp connections. Characteristic of the primary state these exceptional mycelia grow more slowly than do the clamped monosporous cultures (Plate II, Figs. 1 to 3). This is also illustrated in Plate II, Fig. 4, which shows three secondary mycelia of monosporous origin and one primary mycelium derived from one of the exceptional spores all grown on malt agar for 12 days. That these primary mycelia react as true haplonts is proved when they mated, for, in certain pairings, a mycelium bearing clamp connections is produced. We have, then, heterothallism occurring within what appears to be a homothallic species.

Of the 18 monosporous mycelia that remained without clamp connections, only 12 survived. These were maintained in culture for eight months without producing clamp connections. They showed great variation in rate of growth and in the type of colony developed as indicated by Plate II, Figs. 2 and 3. Some grow extremely slowly forming a compact colony with some aerial mycelium. Others grow relatively quickly and look not unlike the secondary mycelia in that they are more diffuse and have little aerial mycelium. The 12 surviving exceptional mycelia were mated in all possible combinations; the results are shown in Fig. 1, page 261. The (+) sign indicates that mating was successful (i.e. that clamp connections developed), the (−) sign indicates mating failure.

PLATE II



Aleurodiscus canadensis showing variation in rate and type of growth of monosporous mycelia on malt agar.

FIG. 1. *Secondary (clamped) monosporous mycelium. After 20 days.* FIGS. 2 AND 3. *Primary monosporous mycelia. After 20 days.* FIG. 4. *Three secondary and one primary monosporous mycelia. After 12 days.*

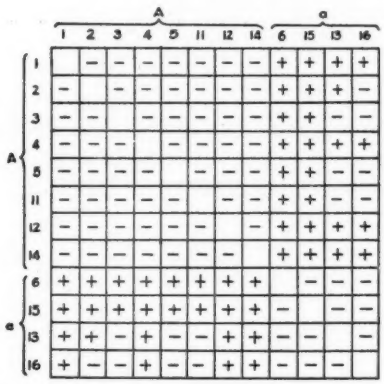


FIG. 1. *Results obtained by pairing, in all possible combinations, 12 haploid monosporous mycelia.*

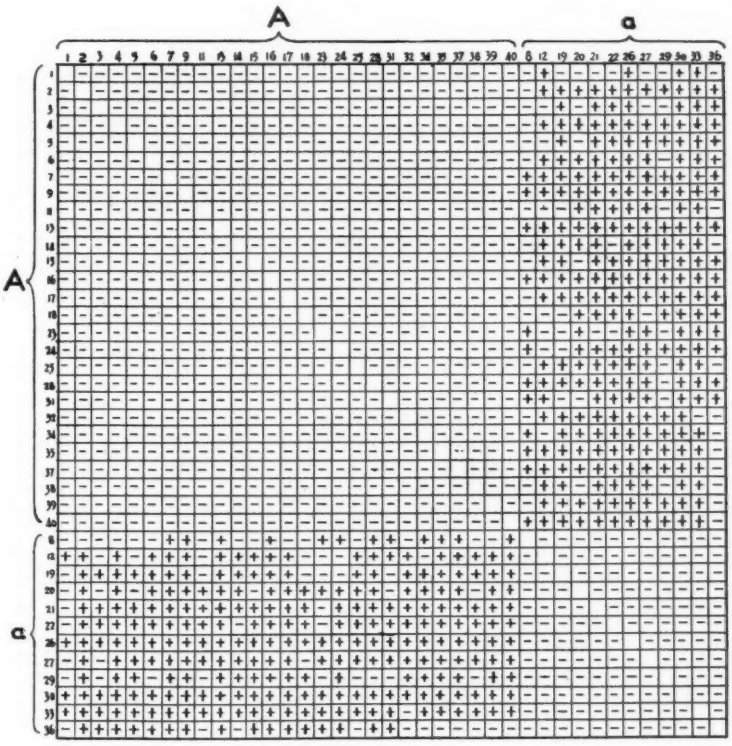


FIG. 2. *Results obtained by pairing, in all possible combinations, 39 haploid monosporous mycelia.*

From Fig. 1 it would appear that the haplonts fall into two groups on the basis of their reaction in mating. To apply a factorial interpretation, there is a single pair of factors governing interfertility, (*A*, *a*). It will be noted that there are a number of exceptions to a strictly bipolar table, as for example crosses 3 × 13, 3 × 16, etc., but the bipolar pattern is unmistakable.

To verify these results a second series of isolations was made in July, 1940. Two hundred and five spore isolations yielded something over 40 unclamped mycelia of which 39 survived. The exact number that failed to develop was not noted. This represents about 19% of the single spore isolations that remained unclamped and survived as compared with 5% in the first series. Pairing the 39 unclamped mycelia in all possible combinations gave essentially the same results as were obtained from the first series (Fig. 2, page 261).

From Fig. 2, the bipolar pattern is evident but here again there are a considerable number of exceptions where pairings have failed. The possible reasons for these exceptions will be discussed later. The primary mycelia used in the second set of pairings were maintained in culture for over one year and remained unclamped.

Cytological Data

In order to determine as far as possible upon what nuclear basis "homothallism" and heterothallism in this species depends, a cytological investigation was undertaken. Cytological observations were confined to the critical stages in the fruiting body up to the point of spore maturity. The nuclear condition of germinating spores and of the vegetative mycelium was not observed.

Nuclear counts in the subhymenial tissue are difficult to make from sections. However, the constant association of nuclei in pairs is characteristic. The division of a dikaryon as seen in the parallel orientation of the spindles with one of the daughter nuclei in the clamp connection is unmistakable (Plate III, Fig. 1).

The young basidia are binucleate (Plate III, Fig. 2). At this stage the nuclei appear as small darkly-staining bodies vertically arranged in the basidium. The early stages of karyogamy could not be found and when first observed the fusion nucleus is already well formed lying in the lower

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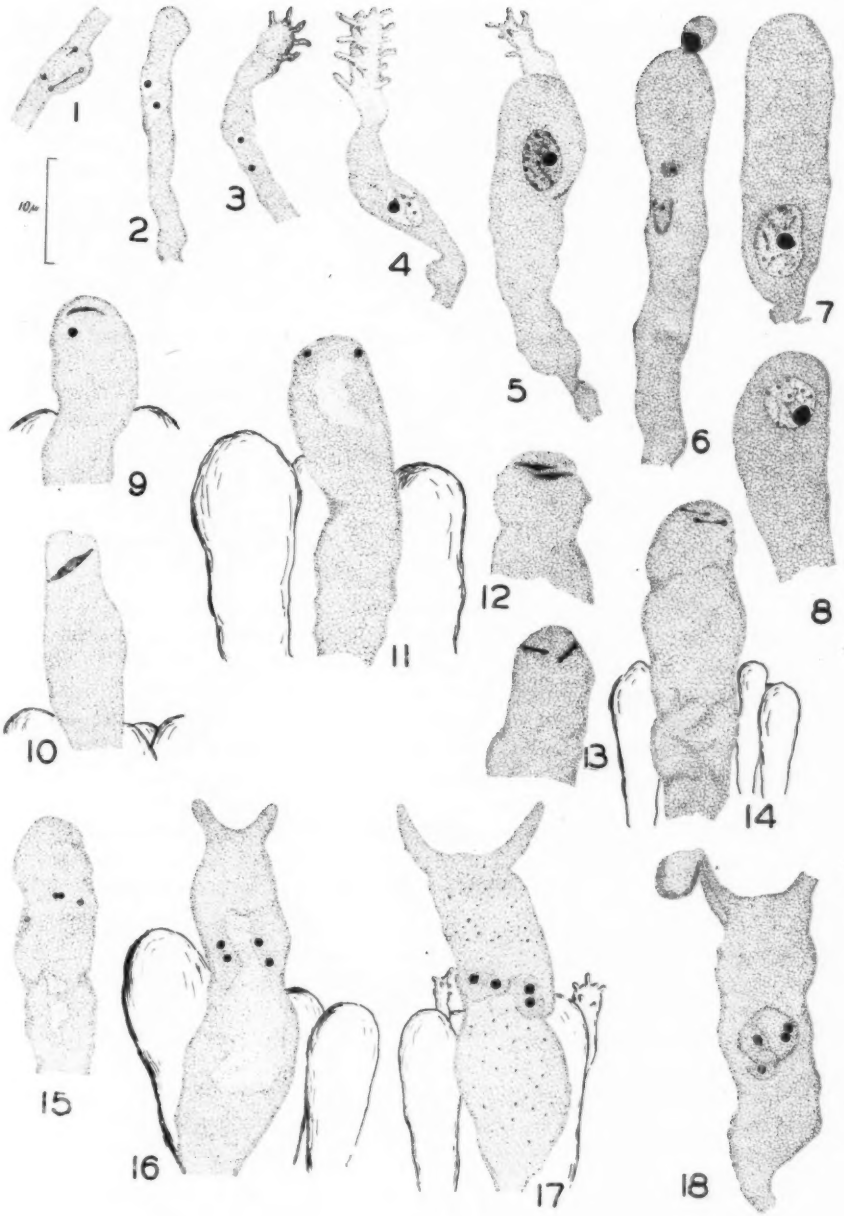
EXPLANATION OF PLATE III

Cytology of Aleurodiscus canadensis.

FIG. 1. *Dikaryon of subhymenial hypha undergoing conjugate division in association with a clamp connection.* FIG. 2. *Young binucleate basidium.* FIG. 3. *Binucleate penicillate paraphysis.* FIGS. 4 AND 5. *Fusion nucleus in penicillate paraphysis.* FIG. 6. *Binucleate moniliform paraphysis.* FIG. 7. *Fusion nucleus at the base of the basidium.* FIG. 8. *Fusion nucleus has migrated to the top of the basidium preparatory to undergoing division.* FIG. 9. *Metaphase of the first division, the nucleolus still persisting.* FIG. 10. *Anaphase of the first division, three chromosomes are visible, also the centrosomes.* FIG. 11. *Telophase of the first division.* FIGS. 12 TO 14. *Second division.* FIG. 15. *Young four-nucleate basidium.* FIGS. 16 AND 17. *Four-nucleate basidia showing development of the sterigmata.* FIG. 18. *Four-nucleate basidium with young spore attached.*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portion of the greatly enlarged basidium. The nuclear outline is well defined while a single large nucleolus and several chromatic granules are visible (Plate III, Figs. 7, 8). These chromatic granules may well be chromosomes becoming visible during this premeiotic phase.

The penicillate paraphyses are at first binucleate (Plate III, Fig. 3). The nuclei fuse and a well developed fusion nucleus is formed (Plate III, Figs. 4, 5), but does not undergo further development. Ritchie (41) has noted that nuclear fusion occurs in the cystidia of *Russula emetica* (Schaeff.) Pers. Whelden (57) reports that in the cystidia of *Peniophora livida* Fr.[1], the nuclei go through one division following fusion, exactly like those in the basidial nuclei. Although no division figures have been found in the penicillate paraphyses there can be no doubt of the presence of the fusion nucleus. The moniliform paraphyses, which are perhaps gloeocystidiate in nature, are also initially binucleate (Plate III, Fig. 6) but nuclear fusion could not be established.

The basidia undergo considerable development during the fusion nucleus stage. Judging by the frequency with which the fusion nucleus is encountered in the preparations this stage is of long duration. The fusion nucleus migrates toward the apex of the basidium and the chromatic granules become more discrete (Plate III, Fig. 8).

The earliest stage of meiosis that was observed shows the spindle already well formed, crowded into the apex of the basidium, and in a transverse position (Plate III, Fig. 9). The nucleolus persists but is much reduced in size and often becomes beaked on one side. The chromosomes do not become distinguishable until anaphasic migration begins. The basidium has become elongated, extending beyond the general hymenial level. Plate III, Fig. 10 shows an early stage in the migration of the chromosomes to both poles of the obliquely transverse spindle. Centrosomes can be seen at either end of the spindle close to the walls. At the close of anaphase of the first division the chromosomes are closely crowded into groups but further telophasic organization does not occur (Plate III, Fig. 11).

In the second meiotic division the two nuclei undergo simultaneous division, with the spindles usually parallel and confined to the apical portion of the basidium (Plate III, Figs. 12 to 14) though this is not always the case. Plate III, Fig. 13, shows the spindles obliquely arranged. The four daughter nuclei are at first very small and are to be seen widely separated in the basidium (Plate III, Fig. 15). As they enlarge and migrate toward the middle of the basidium they frequently appear to be paired (Plate III, Figs. 16, 17). The sterigmata first appear during the downward movement of the nuclei in the basidium (Plate III, Fig. 16). Plate III, Fig. 17 shows the

[1] *It would appear to be quite certain that the species studied by Whelden was not P. livida Fr. in which gloeocystidia are not developed and in which the spores and cystidia are much smaller than in the species with which he worked. Whelden does not mention whether the cystidia were heavily encrusted. If they were, the species may have been P. pubera (Fr.) Sacc. or possibly P. tenella Burt.*

sterigmata elongating and the appearance of the extranucleolar organization of the nuclei. By the time the nuclei have reached the middle of the basidium they are well developed and have increased considerably in size (Plate III, Fig. 18; Plate IV, Fig. 1). Their close grouping makes it difficult to distinguish the outline of individual nuclei. The sterigmata are now fully formed and young spores begin to enlarge. Plate IV, Fig. 1, shows a three-spored basidium with the tetracyte nuclei at this stage. Nuclear migration toward the top of the basidium begins before the spores are fully formed (Plate IV, Fig. 2). All four nuclei move upward together. In Plate IV, Fig. 3, one of the nuclei is seen entering the sterigma and becoming greatly constricted. Prior to migration into the still immature spore the nuclear outline becomes obscure. Plate IV, Fig. 4, shows a much distorted basidium with only one sterigma visible but the actual migration of the nucleus into the spore is to be seen. It is believed that only the nucleolus is visible at this stage and that it becomes constricted and elongated only as it passes into the spore. No cytoplasmic strands have been observed at any stage of nuclear migration as described by Levine (35), Maire (37), and Stork (52). As the spores enlarge and the cytoplasm of the basidium passes into them, the basidium becomes progressively vacuolate from the base. When the spores are fully mature the basidium is practically empty except for the cytoplasm that remains in the sterigmata (Plate IV, Figs. 5 to 7). No evidence of residual nuclei has been found in either two- or three-spored basidia.

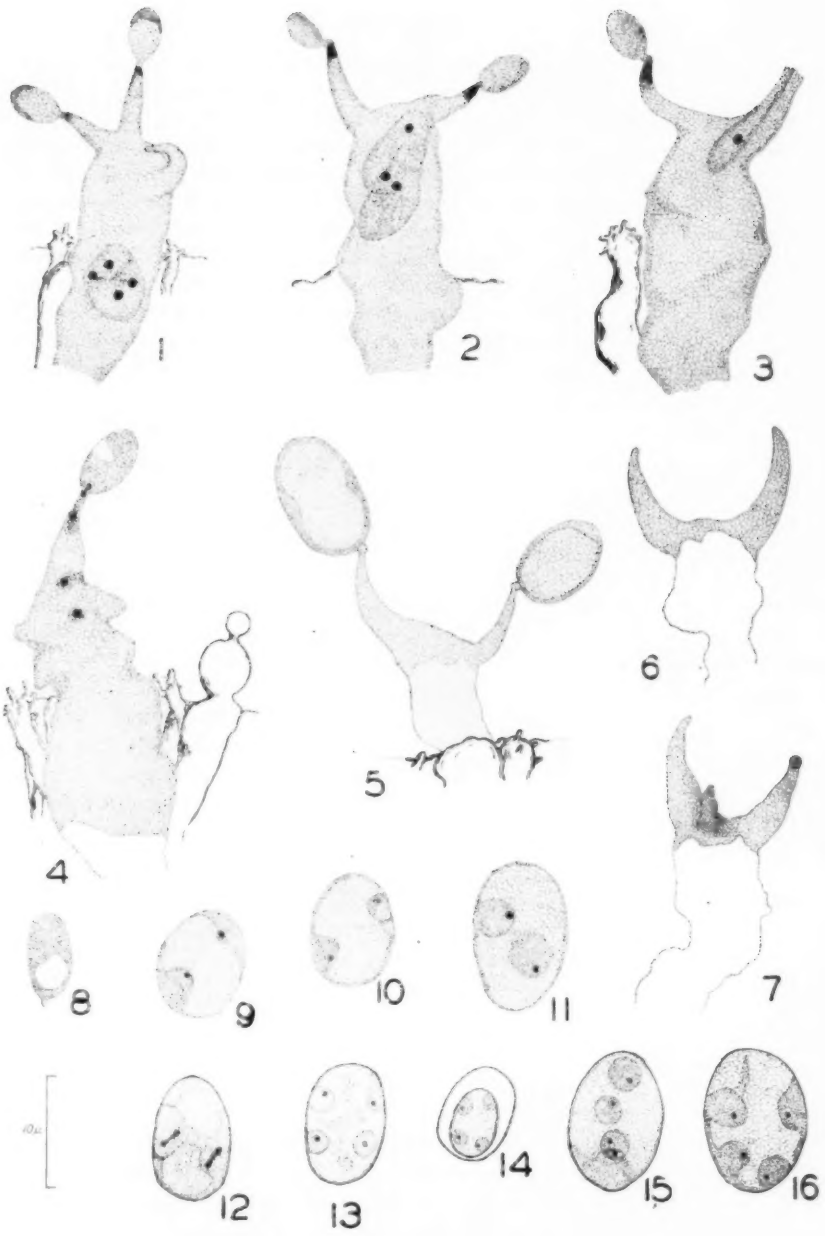
The stages of spore development are illustrated in Plate IV, Figs. 8 to 16. In all cases observed the young spores are at first binucleate (Plate IV, Figs. 8 to 11). These nuclei undergo a single division (Plate IV, Fig. 12) so that the mature spore contains four nuclei (Plate IV, Figs. 13 to 16). It is likely that at this stage the spores are discharged.

No observations on nuclear development in one-spored basidia have been made. It is conceivable that in these the usual four basidial nuclei are produced and that the single spore receives one nucleus. In this event the spore might be expected to give rise to a haploid mycelium. If two (or more) nuclei migrated to the spore it would likely develop a diploid mycelium, provided of course that the nuclei were not alike for interfertility factors. It will be recalled from the cytological review that Ruhland (43) found that in one-spored basidia of *Hydnangium carneum* all four nuclei resulting from meiosis in the basidium passed into the single spore.

EXPLANATION OF PLATE IV

Cytology of Aleurodiscus canadensis.

FIG. 1. *Three-spored basidium in four-nucleate stage.* FIGS. 3, 4. *Migration of the nuclei to the spores.* FIGS. 5 TO 7. *Mature basidia showing vacuolation.* FIGS. 8 TO 11. *Binucleate spores.* FIG. 12. *Nuclear division in the spore.* FIGS. 13 TO 16. *Four-nucleate spores.*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Discussion

From the cytological data presented it is apparent that in the two-spored basidia of *Aleurodiscus canadensis* the nuclear development, up to the point of distribution of the tetracyte nuclei, is the same as in heterothallic four-spored Hymenomycetes. Although actual migration and final disposition of the four nuclei is not completely proved by cytological observation alone, when considered in the light of the cultural results, there can be little doubt that each spore ordinarily receives two nuclei containing allelomorphic factors for interfertility. It has been found that probably 80 to 90% of the spores develop a dikaryotic mycelium directly and that where a spore presumably receives but one nucleus, it produces a haploid mycelium that, in compatible mating with other haplonts, will develop a dikaryotic mycelium. There would appear to be some mechanism that is responsible for the distribution of unlike nuclei to each spore. Some indication of this is to be noted where the basidial nuclei prior to migration are sometimes associated in pairs.

The exceptional haploid spores might conceivably originate in one of several ways. In a two-spored basidium, if instead of receiving two unlike nuclei, each spore received two like nuclei, at least in so far as interfertility factors are concerned, the spores would then be functionally haploid although originally binucleate. No data supporting such a distribution are reported for two-spored Hymenomycetes. In four-spored Ascomycetes, which represent a parallel situation, one such case, in *Pleurage anserina*, has been noted by Ames (2). One presumably binucleate ascospore failed to produce perithecia when grown in culture and in mating this culture with cultures derived from adjacent, dwarf, uninucleate spores, it reacted as a haplont. A suggestion that a similar condition exists in *Neurospora tetrasperma* has been made by Seaver (49) since, in one series of cultures that she studied, 10% of the normal (binucleate) spores did not produce perithecia. It is possible that these binucleate spores, instead of containing two like nuclei, may have contained two unlike nuclei, one of which was non-functional owing to sterility factors.

If the distribution of the four basidial nuclei was unequal in a two-spored basidium, one spore might receive three nuclei and the other spore only one. This possibility has been reported by Ruhland (43) in *Hydnangium carneum*. As a third possibility, each spore might receive only one nucleus and the residual nuclei would degenerate in the basidium. This has been found in a number of species in which the spore normally receives two nuclei, e.g. *Galera triscopa* Fr., *Psalliota campestris* L., *Mycena debilis* Fr., and *Mycena hiemalis* (Osbeck) Fr. (10). There has been nothing in the study of the nuclear condition of *Aleurodiscus canadensis* that suggests any of these possibilities. Sass (47) believes that in the bispored form of *Coprinus ephemerus*, etc., the exceptional haplonts arise as the result of occasional spores receiving one nucleus. He suggests that these uninucleate spores are produced on two-spored basidia in which each spore receives one nucleus and the two remaining

nuclei are left behind in the basidium. Since in these species no three-spored basidia were observed, Sass did not consider the possibility of distribution in such basidia.

Theoretically uninucleate spores might be produced on three-spored basidia by (*a*) the migration of one nucleus to each spore and the degeneration of one nucleus in the basidium or (*b*) the migration of two nuclei to one spore and one nucleus to each of the other two spores. From the evidence obtained on the nuclear condition in three-spored basidia of *A. canadensis* it is known that four basidial nuclei are produced as in the two-spored basidia and, since in no basidia have residual nuclei been observed, it is logical to assume that the latter possibility (*b*) is the more likely. The nuclear sequence described in (*a*) has been suggested by Buhr (10) for the two-spored *Pholiota erebia*, but this was purely speculative. As far as is known, the plan in (*b*) has not been suggested before. Whatever their origin there can be little doubt from the cultural results that certain spores give rise to functionally haploid mycelia. It is presumed, therefore, that these haplonts are derived from uninucleate spores produced on three-spored basidia in the manner indicated above in (*b*) and that two uninucleate spores replace one of the binucleate spores of the normal two-spored basidium. This explanation ties in well with the situation in those four-spored Ascomycetes in which two uninucleate dwarf spores replace one of the binucleate normal spores.

In both series of matings of the exceptional haplonts, it will be recalled that, although the tables were essentially bipolar, there was a considerable number of failures where compatible relationships were expected. If a single pair of allelomorphic factors governed interfertility absolutely, such failures should not occur. As has been mentioned, some of the paired colonies grow very slowly and failure to detect the presence of clamp connections where these slow-growing haplonts were used may account for some of these irregularities. But even in matings between some of the relatively fast-growing colonies failures are found. Similar failures have been encountered in four-spored species by other investigators as pointed out by Barnett (3). "Irregularities were found in pairings of monospore cultures . . . . . these were represented by constant incompatibility between certain monospore cultures of different sexual phases. Such irregularities or deviations from the normal polarity have also been reported by Kniep (10) [33][1], Vandendries (21, 22) [53,54], Brunswik (4) [9], and Dickson (7) [17] for various Hymenomycetes. As suggested by Dickson there seem to be present some conditions or additional factors which influence the compatibility of monospore cultures. Vandendries (22) [54] considers that the tendency toward incompatibility is due to 'une deficience des realisateurs sexueles.' Kniep (10) [33] attributes such deviations to quantitative gene changes." Whatever truth may lie in these suggestions, it seems possible to offer a more concrete explanation for the irregularities in *Aleurodiscus canadensis* because of the type of nuclear cycle present in this species.

[1]*The numbers in square brackets are key numbers to references listed at the end of this paper.*

In normal heterothallic species, interfertility or self-sterility factors provide, through hyphal anastomoses between unlike haplonts and the ultimate fusion, in the basidium, of the dikaryon thus established, that the fusing nuclei will be genetically unlike. Heterothallism is essentially a provision for hybridity. We have in heterothallic forms an "open" nuclear cycle and the possibility of wide crossing with resulting heterozygosity and heterosis.

In the two-spored basidium of *A. canadensis* the nuclear cycle is apparently "closed". Of the four basidial nuclei that are the result of meiosis, two nuclei bearing allelomorphic factors for interfertility normally pass into each spore. Thus the dikaryotic or diploid stage, which in a heterothallic species is developed following hyphal fusion between compatible haplonts, arises here directly in the spore. The primary or haploid phase has been entirely suppressed and the closest kind of inbreeding is the result. Even in self-pollinated higher plants there is a limited gametophytic development allowing for the possibility of cross-pollination. Inbreeding tends to produce homozygosity with the deleterious effect of recessive genes, which become effective only in the homozygous condition. In the higher plants where the results of inbreeding are best known, it has been found that loss of vigour and fecundity often occurred. Similar effects have been observed in *A. canadensis*. Some spores abort (Plate I, Fig. 3) or fail to germinate. Some mycelia fail to develop or grow more slowly than others. The recessive deleterious genes may manifest themselves at various stages of development and need not be linked with factors of interfertility. Sass, too, found a similar mortality in the two-spored form of *Coprinus ephemerus*. He states that "the percentage of spore germination is extremely variable, ranging from 5 to 95 per cent" and that of the germinated spores isolated, 12% of the mycelia died in a few days. With reference to the two-spored form of *Galera tenera* he says that "the mortality among germinated spores was rather high", slightly over 20% of the isolations. In explanation of this condition Sass says "we can expect that characteristics other than sex are determined by nuclear phenomena and perhaps by genetic factors. Many spores of *Coprinus ephemerus* f. *bisporus* fail to germinate, and the wide variations in the percentage of germination may have a cytological basis. Examination of living hymenium shows that in a given area the spores are of the same size and there is no evidence of abortion of spores. It was found that the spores become nearly full-sized and sometimes begin to darken before nuclear migration. . . . . Perhaps it is too radical to suggest that a spore may become full-grown and shed without having received a nucleus. It is possible that only certain of the tetracyte nuclei are capable of giving rise to a viable haploid spore." So far only diploid stages in the normal two-spored forms have been considered. The originally haploid spores of *A. canadensis*, which presumably arise on three-spored basidia, offer an escape from the closed cycle and the opportunity for wide crosses. It has been shown that the haploid mycelia derived from such spores will, if mated with compatible haplonts, produce the diploid stage. It may be expected that these exceptional haplonts will show the deleterious

effects that are found in the parental diplonts and that have resulted from continued selfing. The failure to mate when two haplonts with allelomorphic interfertility factors are brought into association might thus be expected.

Sass (47) isolated one set of exceptional haplonts from one fruit body and a second set from another. By selecting the two most "fertile" haplonts from each set (i.e. the haplonts that entered into the greatest number of crosses) and pairing them, he obtained a normal bipolar table. Had he mated less "fertile" haplonts from the two sets it is possible that he may have obtained similar results, the effect of heterosis. As mentioned earlier in this paper Sass's tables of mating of aberrant haplonts in the two-spored forms of *Coprinus ephemerus* and *Galera tenera* showed no bipolar relationship. Only in a very few cases did a dikaryotic mycelium develop following mating. The four-spored form of *C. ephemerus*, was found to be heterothallic and bipolar. Sass's conclusion that probably more than one pair of factors are involved in the mating of "aberrant" haplonts may be incorrect. The cultural results of the present study suggest that had Sass used a larger number of haplonts, the bipolar nature of his tables may have become evident.

If the writer's suggested explanation of the irregularities in pairing exceptional haplonts is at all acceptable, one might expect to find a similar situation to exist in Ascomycetes with reduced spore numbers as in *Neurospora tetrasperma*, *Pleurage anserina*, etc., where the life cycle is essentially the same as in the present material. Such irregularities are not reported in any of these Ascomycetes although Dowding (23) states that of 54 dwarf (uninucleate) spores isolated, altogether 40 germinated. Ascus abortion in *Neurospora tetrasperma* (20, 21, 22), has been attributed to lethal factors that are inherited in normal Mendelian fashion. It may be that irregularities in pairings or failures in germination or mycelial development in these Ascomycetes do occur but have not been mentioned.

Throughout this paper the writer has referred to *A. canadensis* as being "apparently homothallic" since the majority of spores give rise to dikaryotic mycelia without mating. The reference to "apparent homothallism" requires to be enlarged upon. In a heterothallic form the dikaryotic, clamp-bearing mycelium has its origin through hyphal anastomosis between two haploid thalli whose nuclei are of different genetic constitution. The two nuclei thus derived from the two parent haplonts retain their identity and are presumably maintained in association in each cell throughout the development of the diploid thallus by means of conjugate divisions in association with clamp formation. The nuclear phenomena underlying homothallism in a form such as *Coprinus sterquilinus* Fr. have been disclosed by the investigations of Brunswik (9), Buller (11, 12), and Sass (48). In the homothallic *Coprinus sterquilinus* the nuclear cycle is closely parallel to that of a heterothallic species. The spore receives a single nucleus that has resulted from reduction division in the basidium. A dikaryotic mycelium with clamps is soon produced as this originally uninucleate spore develops, but since there is no justification for the assumption that segregation can occur other than at

reduction division, the two associated nuclei of the dikaryon must be sister nuclei of identical genetical constitution. The subsequent fusion and reduction division in the basidium, therefore, can have no genetical significance. It is conceivable, therefore, that the association might be omitted entirely as in haploid parthenogenetic forms. The time of origination of this association of sister nuclei might be delayed until just before fusion, or might be moved backward to the spore. The explanation of the erratic position of nuclear association and the occurrence (or absence) of fusion and reduction in truly homothallic forms that seems most reasonable is that such forms are derived from heterothallic species in which all the processes presumably have a phylogenetic origin and certainly a genetic significance (28). In homothallic forms the genetic significance of these processes ceases to be operative and, while the influence of long phylogenetic history is evident in the persistence of an apparently normal sequence in such a form as *Coprinus sterquilinus*, this influence is broken down in others. Hence variations from the normal nuclear sequence of the typical heterothallic parental types occur in the homothallic derivatives.

It is also evident that it is not correct to refer to such forms as *Aleurodiscus canadensis*, *Neurospora tetrasperma*, *Pleurage anserina*, etc., as homothallic unless homothallism is defined simply as the ability of an organism to carry out its full life history from the activity of a single spore. Such a definition is inadequate and incorrect in the face of definite proof that each spore contains two nuclei of different genetic constitution. In this study, for the first time in the Basidiomycetes, a factorial basis for this genetic difference has been shown to exist. The term homothallism in the higher fungi should be restricted to those fungi where the full life cycle may result from the development of a single uninucleate spore. Buller (13), although recognizing the difference between a homothallic species such as *Coprinus sterquilinus*, which begins its life cycle from a uninucleate haploid spore and which he designated as "haplomonoecious homothallic", and those species in which the spore is originally binucleate, containing allelomorphic factors for interfertility, and referred by him as "heterokaryotic or miktohaplontic homothallic", does not attempt to restrict the term homothallism to fungi of the first type.

Acknowledgments

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